TRANSFUSION

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Editorial

Rags to Riches

Looks can be disarmingly deceiving. The mature mammalian erythrocyte, incapable of dividing without a nucleus, was for a long time viewed as a hapless sac of hemoglobin designed for passively transporting oxygen and carbon dioxide. Shortly after it was discovered that sodium citrate could safely be used to keep blood fluid for transfusion, the addition of glucose was found to retard the appearance of hemolysis *in vitro*. The needs of the then lowly red cell were of little concern for the next quarter of a century. During World War II red blood was needed in combat. Much attention was lavished on the red cell, and it was learned that in suitable containers it could be kept fit for transfusion for as long as three weeks if temperature, *p*H and glucose concentration were just right. The high level of 2,3-diphosphoglycerate (2,3-DPG), unique to the erythrocyte, described two decades before was rediscovered and the importance of adenosine triphosphate (ATP) was realized before adequate means of quantitation were at hand.

Since the end of World War II the number of contributions relating to the metabolism of the erythrocyte have been so numerous that space limitations prohibit specific references to any here. Much has been learned by studying hemolysates, and the remarkable effects of purine ribosides were first demonstrated in such a system. More recently it was discovered that the purine ribosides (adenosine, inosine, guanosine) are capable of rejuvenating stored red cells by mediating the regeneration of their depleted supply of ATP and 2,3-DPG. Unfortunately the high hope for the practical application of an ACDInosine preservative has not materialized.

The two papers by Bishop in this Journal (see pages 349, 355) utilize the elegant technic of ion exchange chromatography for studying the nucleotides of the erythrocyte. He suggests that "The changes in the nucleotide pattern of stored blood are so characteristic that they may be used to date stored blood or to compare different methods of storage." The problem is complicated by the fact that as yet correlation between the nucleotide pattern and *in vivo* survival of red cells has not been established.

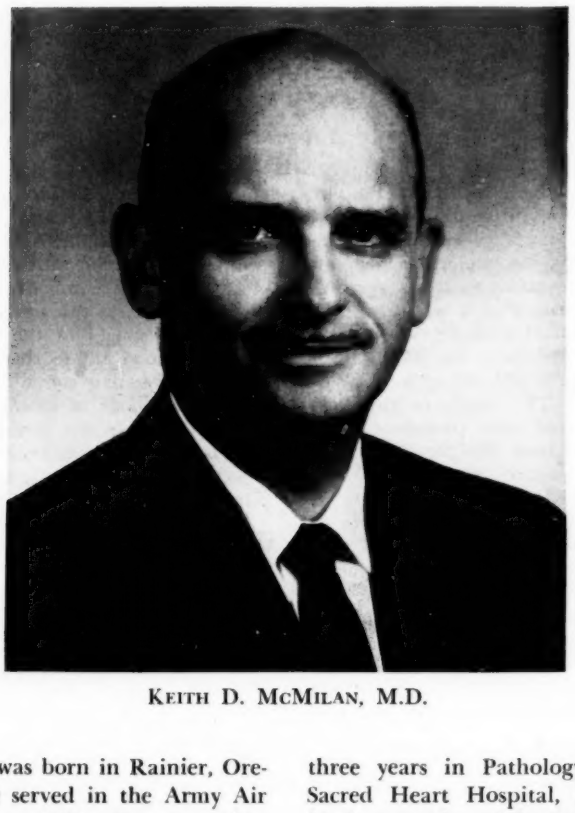
A new door was opened by the observation that the hemolytic anemia associated with primaquine administration is related to the glutathione content and the stability of glutathione in the red cells. The primary, genetically determined, defect is probably deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) activity. It is responsible for the hemolytic complications occasionally encountered with many drugs and the fava bean.

Now the Embden-Meyerhof and the hexose monophosphate pathways are favored haunts of researchers. Suffice it to say that the majority of the kinases, dehydrogenases, isomerases and mutases involved in these pathways of glycolysis have been found to be present in erythrocytes.

The erythrocyte can be made to hibernate for years at temperatures of −80 C. to −120 C. if glycerol is added to bind water. Very rapid freezing at the temperature of liquid nitrogen (−196 C.) in the presence of glucose or glucose and lactose is also possible. Under

these conditions all metabolic activity is curtailed and if the physical conditions are suitable it may be possible to store erythrocytes indefinitely. Resort to such drastic measures seems rather crude. Perhaps, when we have learned more about the rich bag of tricks concealed within the red cell, prolonged periods of suspended animation will be achieved by blocking metabolism at just the right point. T. J. GREENWALT, M.D.

New President - A.A.B.B. 1961-62



KEITH D. MCMILAN, M.D.

DR. MCMILAN was born in Rainier, Oregon in 1908. He served in the Army Air Corps from 1929 to 1932. One year after graduation from the University of Oregon in 1938, he married Barbara E. Hollis.

In 1941 Dr. McMilan received his M.D. degree from the University of Oregon Medical School; service with the Army Medical Corp followed (1941-45). He served his internship at Presbyterian Hospital, Los Angeles, California (1941-42) and spent three years in Pathology (1946-1949) at Sacred Heart Hospital, Eugene, Oregon. He is a member of the American Society of Clinical Pathologists and the International Academy of Pathology.

Dr. and Mrs. McMilan with their two children, Amelie Ann and Hollis K., reside in Eugene, Oregon where the Doctor is engaged in the practice of Pathology and serves as Medical Director of the Lane Memorial Blood Bank.

Changes in the Nucleotides of Stored or Incubated Human Blood*

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Human blood was stored in ACD solution (acid citrate dextrose) for up to eight weeks at 4 C. or up to 72 hours at 37 C. The changes in the blood nucleotides were followed serially by fractionation on Dowex-1-formate resin. The breakdown of ATP in aging blood followed the pattern, ATP→ ADP→ AMP→ IMP→ hypoxanthine, causing a steady decrease in ATP concentration and a continuing rise in hypoxanthine concentration with a transient rise and fall in the intermediate nucleotides. The reactions were roughly similar at both temperatures but required eight weeks at 4 C. as against only three days at 37 C. The changes in the nucleotide pattern of stored blood are so characteristic that they may be used to date stored blood or to compare different methods of storage. The exact correlation between the nucleotide pattern of stored blood and red cell survival is not known, but it generally is accepted that both are related to the length of time and storage conditions of blood.

FRESHLY-DRAWN blood contains a variety of nucleotides, distributed in a characteristic pattern.[3] When such blood is allowed to stand under any conditions, the nucleotide pattern begins to change. Gabrio, *et al.*,[5] presented typical column chromatograms of blood at 0 days and 28 days of storage, showing the loss of ATP** and the increase in AMP and IMP. Serial changes in the nucleotides and other phosphate compounds of blood incubated at 37 C. for up to 24 hours were reported by Mills and Summers.[10] Changes in the phosphate compounds of human blood stored under blood bank conditions for up to 62 days were studied by Bartlett and Barnet.[1]

The present paper describes in detail the changes in nucleotides in the blood of two healthy males when the blood was stored under blood bank conditions for up to eight weeks or incubated at 37 C. for up to 72 hours. These data can be used to date blood stored under these conditions and also to compare other storage systems to the ACD system. In addition, the pathway of nucleotide breakdown in blood is delineated by the time relationships in the rise and fall of the intermediates.

The human red cell has only one source of energy—glycolysis. As long as the red cell remains viable and continues to glycolyze, it will be able to synthesize high energy compounds such as ATP. Hence, the nucleotide pattern of blood will be an indicator of red cell viability. The relationships between red cell viability and red cell survival under transfusion conditions are as yet poorly defined but the present nucleotide fractionation method can certainly be used as a measure of red cell viability and as an approximate index of red cell survival.

Methods

Blood was drawn from two normal males and expressed into siliconized flasks containing an appropriate amount of ACD solution.† Aliquots of 10 ml. each were with-

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** The following abbreviations are used: ATP, ADP, AMP = adenosine tri-, di-, and mono-, phosphate; GTP = guanosine triphosphate; IMP = inosine monophosphate.

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† Acid citrate dextrose, National Institutes of Health, Solution A.

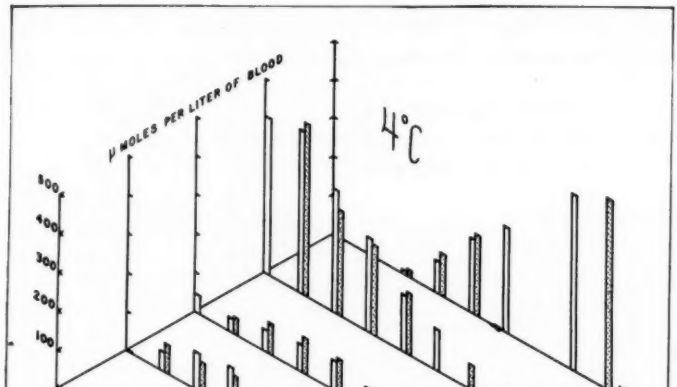

FIG. 1. Changes in the distribution of adenine and hypoxanthine nucleotides and free hypoxanthine in blood of two normal male subjects. Blood was stored in ACD solution at 4 C. Compounds are abbreviated as noted earlier in paper except Hx = hypoxanthine.

drawn, securely capped, and stored in a blood bank refrigerator or placed in a water bath at 37 C. The latter samples were swirled several times a day to resuspend the red cells while the former were swirled once a week. Parallel samples, which were agitated more frequently, gave essentially similar nucleotide distributions; thus, frequent or continuous agitation was presumed to be unnecessary. In the 4 C. series, samples of both subjects' blood were taken at the same time intervals up to four weeks but in the later weeks and also in the 37 C. series, the times were staggered for the two subjects' blood so that more intervals could be studied. Strict aseptic technic was observed in handling all samples and occasional samples submitted to our Department of Bacteriology were adjudged sterile. Slight hemolysis occurred in older samples in both series, a situation commonly encountered when blood from blood banks becomes outdated. At the indicated times, the appropriate samples were cooled in ice and 10 ml. of 10 per cent ice cold trichloracetic acid were added. The handling of the extraction and subsequent fractionation of the nucleotides were as previously described.[3] The wash from the Dowex-1x10-formate column was passed through a column containing Dowex-50-H+ and the column thoroughly washed with water. Elution with 1.0 N HCl was then begun and hypoxanthine was eluted after about 150 ml. of acid had flowed through the column. No other purine, pyrimidine, or nucleoside was seen in the wash although appropriate elution conditions were instituted on several occasions. Subsequent studies have shown that inosine appears under certain storage conditions but that its presence is transient. It is eluted ahead of, and usually separate from, hypoxanthine. The area under the hypoxanthine peak (measured at 260 mμ, as in the nucleotide system) was multiplied by the factor 68.0 to convert the sum of the absorbancies of the 5 ml. fractions

(minus background due to absorbancy of formate, etc.) to micromoles of hypoxanthine per liter of whole blood. The calculation is derived in the same manner as that described previously,[3] using 7.3 x 10^3 as the molar absorbancy of hypoxanthine in 1.5 N HCl at 260 mμ.[4]

Results

Storage at 4 C.: The results of these experiments are shown in Figure 1. In freshly drawn blood, ATP is the major nucleotide. There is about 1/10 as much ADP as ATP, and AMP is only just detectible. There is no IMP, and hypoxanthine is almost undetectible. Other nucleotides and dinucleotides such as GTP, DPN, and TPN are present in whole blood but they are not discussed in this paper because the important sequence of events is related to ATP breakdown, which goes as follows:[2]

ATP→ ADP→ AMP→ IMP→
(inosine)→ hypoxanthine

An early change noted in the nucleotide pattern of stored blood is a decrease in the ATP concentration and an increase in the ADP/ATP ratio, followed by an increase in the ADP and AMP concentrations. The appearance of IMP and appreciable quantities of hypoxanthine soon follows. Since ATP disappearance and hypoxanthine appearance are at alternate ends of the reaction scheme, it is apparent that the changes in these compounds should be regular and unidirectional, *i.e.*, decrease of ATP and increase in hypoxanthine. However, the concentrations of the intermediates such as ADP, AMP, and IMP first increase, then decrease. The exact relationships among all these nucleotides at any particular time are obviously related to several reaction velocities or equilibria. The complete nucleotide pattern at any particular time, however, is quite reproducible, as shown by the fact that the data in Figure 1 are based on two subjects' blood. Many other blood storage systems have been studied in this laboratory and the trends described here in the nucleotide patterns are quite general, but the exact

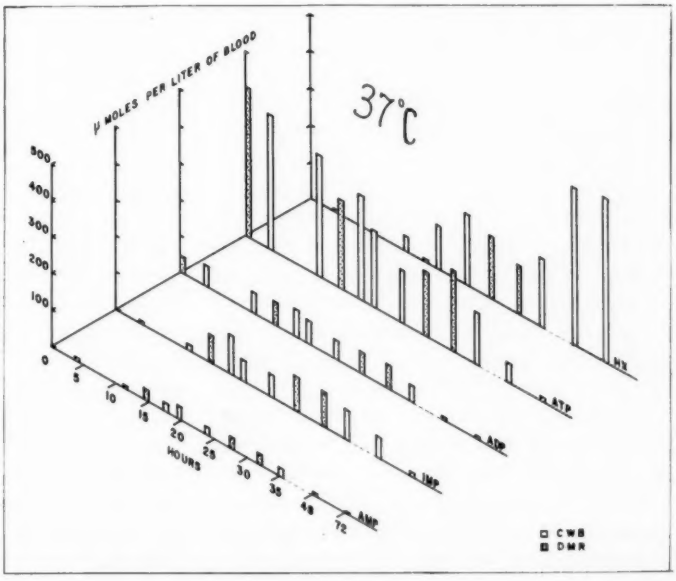

FIG. 2. Same as Figure 1 except that blood was incubated in ACD solution at 37 C.

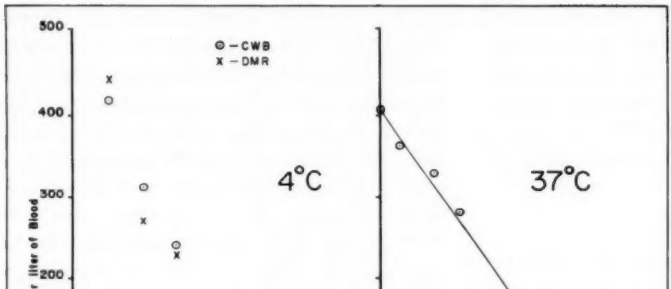

FIG. 3. Decline in ATP concentration with time. Replotted from data of Figures 1 and 2.

values are variable. For example, one blood might start with an ATP concentration of 500 micromoles per liter while another might have only 400 micromoles of ATP per liter. Yet, in each the ADP/ATP ratio would be about 1/10 and there would be no IMP and little hypoxanthine. Upon storage, the characteristic changes would occur in both bloods but the relative patterns rather than the absolute values would be comparable. This poses no problem in evaluating blood storage systems because we routinely draw replicate blood samples from the same subject and follow the blood nucleotide patterns serially. Which system gives rise to more rapid deterioration of the nucleotide pattern can be easily judged, and the comparison is based on concentration changes in several compounds.

Incubation at 37 C.: Studying the blood nucleotides under actual blood bank storage conditions is the only way to discover the exact changes that take place under those circumstances, but because of the length of time involved (eight weeks in these experiments), a systematic consideration of storage systems would be very time-consuming. For this reason we re-ran our first set of experiments at 37 C. instead of at 4 C. to see if the changes in the blood nucleotides would be the same. From Figure 2 it can be seen that the overall changes are the same, namely, ATP disappearance, hypoxanthine formation, and a transient increase and decrease in the intermediates, *i.e.*, ADP, AMP, and IMP. The consistency of agreement between the two subjects' blood, however, was poorer than previously. This suggests that diffusion or some metabolic reaction, which was adequate at the 4 C. temperature, might become rate-limiting as metabolism speeded up.

In general, the blood nucleotide pattern in eight weeks (56 days) at 4 C. was more or less equivalent to that at 72 hours (three days) at 37 C. This means that the overall speed of the changes was approximately 19 times as fast at 37 C. as at 4 C. If one assumed a doubling of reaction rate with a 10 C. temperature rise, as is commonly done with chemical reactions, then the rate at 37 C. should be between eight and 16 times that at 4 C., a figure at least of the same magnitude as in these experiments.

One point that may or may not have further significance was the rate of disappearance of ATP at the two temperatures. In Figure 3 these data are plotted. At 4 C. the decline of ATP was essentially logarithmic while at 37 C. it was linear (in one subject only, insufficient data were available from subject DMR for a good plot). In general, one would associate the logarithmic curve with a substrate-dependent or first order reaction, while a linear fall would be associated with a substrate-saturated or zero-order reaction. The subtleties of this situation were not explored since this study was designed to lay the groundwork for nucleotide changes in blood stored in various systems and under various conditions. In general, however, it seems fair to state that the changes in blood nucleotides stored in ACD solution at 4 C. or 37 C. are essentially the same, at least from the point of view of evolving and testing storage systems. Other workers have reached similar conclusions.[11]

Discussion

In the experiments reported here, the sum ATP + ADP + AMP + IMP + hypoxanthine was constant for a series of samples on a subject. The same constancy was noted by Jørgensen[6] except that he also included xanthine in his sum. Xanthine would not arise directly from hypoxanthine by the action of xanthine oxidase since this enzyme is absent in blood (see 3 and its references) and also because this enzyme would not only oxidize hypoxanthine to xanthine but xanthine to uric acid as well. Hence, the constancy noted in the present results and by Jørgensen could not obtain. There is the possibility of xanthine arising from xanthylic acid by the action of xanthylic acid pyrophosphorylase. Evidence for the conversion of IMP to XMP has been presented both for human blood[2] and rabbit erythrocyte.[8] Another possible origin of xanthine is from the small amount of GTP present in blood.[3] Jørgensen[7] has shown that xanthine can be formed by human blood from added guanine, guanosine, or xanthosine and these could probably be derived from GTP under certain conditions. If xanthine were derived from GTP rather than from ATP, then it should not be included in the sum: ATP + ADP + AMP + IMP + inosine + hypoxanthine, but if the xanthine were derived from IMP then it should legitimately be included as an ATP breakdown product. For non-isotopic studies, the question is academic because the amount of xanthine expected would likely fall within the experimental error of the methods used.

In preliminary studies on rabbit blood, we have observed that the sum of ATP + ADP + AMP + IMP + hypoxanthine was not constant. This suggests the presence of xanthine oxidase in rabbit blood or activity of alternate pathways such as those just mentioned. Evidence for the former lies in the fact that the hypoxanthine does not continue to increase as deterioration proceeds. In any event, the concentration of hypoxanthine in rabbit blood cannot be used as a measure of deterioration of the nucleotides of rabbit blood as it can be in human blood.

The sequence of breakdown of ATP in human blood appears to be well established from the above and other studies[2] to be ATP→ADP→AMP→IMP→hypoxanthine. The present author prefers to consider the progression of these reactions as deterioration of the nucleotide pattern. It is generally recognized that this type of deterioration and a decrease in red cell survival do progress together as human blood is held in storage longer. However, it cannot be said that the level of ATP can be used to predict red cell survival, since transfused red cells may retain some ability to regenerate ATP when their environment is restored to normal. On the other hand, there is probably some point of no return,

and its characteristics may be derived by correlation of the present type of study with equally detailed studies of red cell survival. It might be noted in passing that in certain circumstances the measurement of the increase in inorganic phosphate or the decrease in readily-hydrolyzable phosphate could be substituted for the much more exacting nucleotide fractionation as measures of red cell viability.

Acknowledgments

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Factors in the *In Vitro* Maintenance of the Nucleotide Pattern of Whole Human Blood*

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The effects of various glycolytic inhibitors on the relatively stable nucleotide pattern of freshly drawn heparinized human blood, incubated for two hours at 37 C. was observed. Fluoride, iodoacetate, arsenate, or 2-deoxyglucose caused a similar high-energy nucleotide breakdown as observed on hemolysis, viz., rapid disappearance of ATP, GTP, ADP, with simultaneous increase in AMP and IMP. The maintenance of a normal blood nucleotide pattern *in vitro* thus depended on cellular integrity and a source of energy. The addition of compounds believed to enhance or inhibit the pentose shunt pathway had no effect on the blood nucleotide pattern. This is interpreted to mean that under essentially *in vivo* conditions, the major source of energy for the red cell is derived from the Embden-Meyerhof cycle.

WHEN freshly drawn heparinized human blood is incubated at 37 C. under 95% 0_2—5% CO_2, or 95% N_2—5% CO_2, its characteristic nucleotide pattern, particularly with respect to the high-energy compounds ATP, ADP, and GTP, is maintained for two hours.[4] In contrast, the characteristic nucleotide hydrolytic changes associated with loss of high-energy phosphate bonds occurs when whole human blood is stored at 4 C. for several weeks or incubated at 37 C. for several days.[3] The present experiments based on the two hour incubation technic show that the interruption of glycolysis by classical inhibitors such as fluoride, iodoacetate, and arsenate leads to changes in nucleotide patterns that are similar to those seen in blood stored several weeks. Various inhibitors have been used in attempting to elucidate the cause of the deterioration of the blood nucleotides on storage.

Methods

Blood was withdrawn by means of a siliconized syringe and expressed into a siliconized, heparinized flask. Aliquots of 10 ml. were transferred into 40 ml. centrifuge tubes and these were fitted with gassing tubes and placed in a water bath at 37 C. Appropriate concentrations of inhibitors were added and the tubes swirled occasionally to keep the cells suspended.[4] At the end of two hours, the samples were chilled and cold trichloracetic acid was added. The nucleotides were fractionated on Dowex-1-formate resin columns.[5] In most cases, duplicate experiments were performed on blood from two different subjects.

Results and Discussion

When blood is hemolyzed by freezing and thawing twice, over 90 per cent of the red cells are destroyed. Incubation of these hemolysates for two hours produces nucleotide patterns such as that seen in Figure 1. It is apparent that GTP,** ATP, and most of the ADP have disappeared and that AMP and IMP are now in abundance. The maintenance of a normal pattern of blood nucleotides apparently requires integrity of red cell structure and, as will be shown, a source of energy. When the energy sources are cut off, the blood nucleotide pattern deteriorates just as it does after cellular destruction.

Before presenting the experimental results, it is best to review the reactions of

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Preliminary report presented at the meeting of the American Association of Blood Banks, San Francisco, California, August 21-26, 1960.

** Same abbreviations as used in the previous paper.

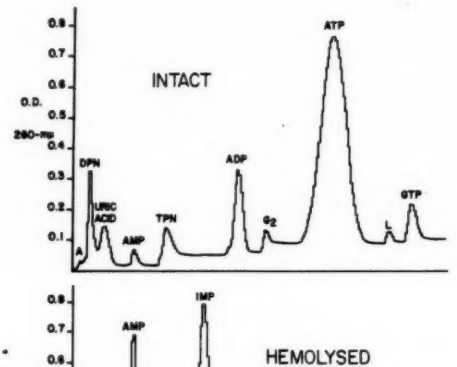

FIG. 1. Nucleotide patterns of whole human blood (Intact) and after hemolysis and incubation for two hours at 37 C. (Hemolyzed).

the Embden-Meyerhof and the pentose shunt schemes of glycolysis. These are outlined in Figure 2, along with the point of action of some of the inhibitors used.

Figure 3 shows the inhibitors that were effective in altering the blood nucleotide pattern (*i.e.*, causing deterioration of the pattern[3]). The first graph in Figure 3 shows that normal blood incubated for two hours without any inhibitor added contains over 400 μ moles of ATP per liter of whole blood. Its ADP level is roughly 11 per cent of this amount, *i.e.*, about 50 μ moles per liter. The amount of AMP is almost negligible, and there is no IMP. There is some GTP and it is included in this tabulation, not because it is related to ATP breakdown,[4] but because its lability mirrors that of ATP and hence serves as an experimental check. The duplicate bars standing together represent results from two different subjects.

The first inhibitor experiments shown in Figure 3 involved p-chloro-mercuribenzoate. This is a classical sulfhydryl inhibitor. It had a slight effect in reducing ATP and GTP and increasing AMP and IMP. It should at least have had an effect on the enzyme, glyceraldehyde-3-phosphate dehydrogenase, just as iodoacetate did in later experiments. That it did not might be attributable to non-penetration of the red cell. A compound of this type, especially one with a heavy metal attached, would probably interact with the red cell membrane and might get no farther. In our experiments, 1.4 x 10^{-3}M PCMB did not cause appreciable hemolysis as evidenced by the persistence of near-normal amounts of ATP, but according to Sheets, *et al.*,[11] and Tsen and Collier,[14] a concentration of 5 x 10^{-4}M PCMB will cause complete hemolysis of human or rat red cells. The ability to cause hemolysis may vary with factors other than concentration, however,

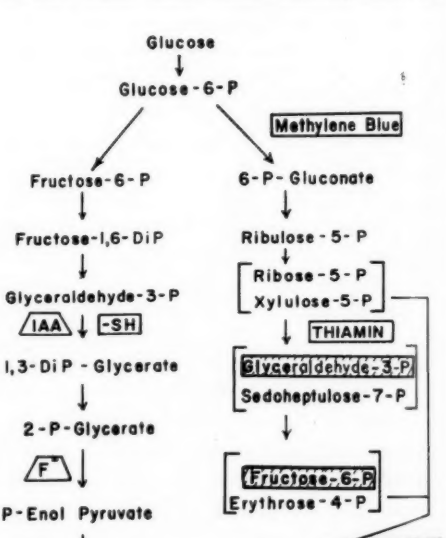

FIG. 2. Outline of the Embden-Meyerhof and pentose shunt schemes of glycolysis. Hatched compounds (fructose-6-phosphate and glyceraldehyde-3-phosphate) feed back into the Embden-Meyerhof scheme. Inhibitors are shown in trapezoids and cofactors in boxes.

because Sheets and Hamilton[12] later reported that at 3.5 x 10^{-3}M, hemolysis was incomplete.

Oxamic acid inhibits lactic dehydrogenase[9] and is a very effective inhibitor of anaerobic glycolysis in Ehrlich ascites tumor cells, among other systems. Inhibition of lactic dehydrogenase in the present system would halt glycolysis unless DPNH could be oxidized through another pathway.

Tolbutamide is a hypoglycemic agent *in vivo*, whose mode of action is still in dispute.[2] In the present system, it seemed to promote the breakdown of some ATP with consequent production of ADP. This effect has been studied more extensively in this laboratory and has been shown to be repeatable but no explanation can as yet be offered for it.

The introduction of 2-deoxyglucose into this glycolyzing system offers a molecule that competes with glucose for metabolic sites. It was effective in the present system in reducing the ATP. It must be remembered that IMP is converted to hypoxanthine in incubated whole human blood[4] and that therefore, IMP concentration need not rise under circumstances of ATP breakdown.

Arsenate is usually thought of as an ion which will form organic esters similar to phosphates but which are more susceptible to hydrolysis. Since ultimate hydrolysis of high energy bonds without a means for trapping the energy by a coupled reaction represents wastage of bond energy, the addition of arsenate should favor the disappearance of di-, and tri-phosphates, which are the high energy compounds in the present system. Such, indeed, was the result. In this situation, as in the previous one, IMP was apparently converted to hypoxanthine.

Iodoacetate is a potent —SH inhibitor, being particularly effective against the enzyme, glyceraldehyde-3-phosphate dehydrogenase.[6] The great increase in AMP at the expense of ADP and ATP is consistent with the need for an energy source to maintain the nucleotide pattern. Cutting off glycolysis via the Embden-Meyerhof cycle foredooms the system to deterioration.

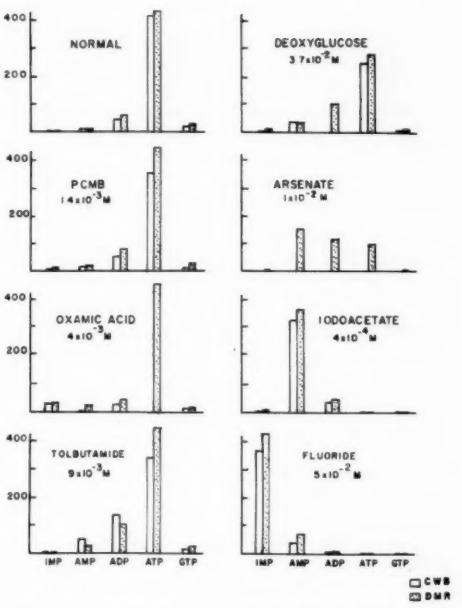

FIG. 3. Amounts of various blood nucleotides when heparinized whole human blood was incubated for two hours at 37 C. under 95%-O_2: 5%-CO_2 with the compounds noted. Duplicate bars indicate separate experiments with two different donors.

Fluoride acts on enolase[15] and hence, also stops the Embden-Meyerhof cycle. Both fluoride and iodoacetate may also interdict the pentose shunt since the products of the shunt feed back into the Embden-Meyerhof cycle at or above glyceraldehyde-3-phosphate. The interesting difference between the nucleotide pattern with iodoacetate and fluoride is the absence of IMP and great excess of AMP in the former situation and almost the reverse situation in the fluoride-inhibited system. This difference may represent the same basic nucleotide deterioration scheme but seen at different relative times in two sys-

TABLE 1. *Compounds Having Slight or Equivocal Effect on the Nucleotide Pattern of Incubated Human Blood*

Compounds	Concentration	Effect
Iproniazid	2.7×10^{-4}M	ADP lowered
Thyroxin	1×10^{-4}M	ATP lowered*
2,4-Dinitrophenol	1×10^{-4}M	ATP lowered*
Benzimidazole	5.9×10^{-3}M	ADP increased

* Single experiments only

tems that were moving at different rates. Serial studies of both systems would show if this is indeed the situation. Alternatively, the rate of deamination of AMP versus the rate of splitting of IMP to hypoxanthine might be differentially affected by the two inhibitors, giving rise mainly to IMP in the one case and AMP in the other. In either case, hypoxanthine formation must be proceeding.

Table 1 lists the compounds that had a slight or equivocal effect on the nucleotide pattern of incubated blood. The slight effect brought about by the added iproniazid is of interest since its action at the cellular level is poorly understood. Thyroxin and 2,4-dinitrophenol are agents known to "uncouple" oxidative phosphorylation. Since only substrate phosphorylation is presumed to proceed in the present system, it is difficult to explain their effect in lowering ATP. However, any interaction between ATP and these compounds might favor net dephosphorylation if a transferred high energy phosphate were more easily hydrolyzed. Benzimidazole is an interesting compound since it is similar to purine except with two ring nitrogens replaced by two carbons. It obviously did not disturb the nucleotide distribution greatly. If it had had a 6-amino, or 6-hydroxy group, perhaps it would have been more effective as an adenine or hypoxanthine analog.

Table 2 lists the compounds having no apparent effect on the blood nucleotide pattern. It has been known for years that methylene blue will increase the oxygen uptake of mammalian erythrocytes.[8] This effect results from increased metabolism via the pentose shunt. Since the nucleotide pattern of the incubated blood was not affected in any way, it was concluded that under the present experimental conditions, the pentose shunt and nucleotide metabolism were not intimately related.

Thiamine is required for transketolase in the pentose shunt.[13] Supplying extra amounts of thiamine or adding thiamine inhibitors such as pyrithiamine or oxythiamine might have been expected to influence the blood nucleotide pattern. None of these had any effect. Poor penetration of the cell can always be invoked as a reason for no effect in cases such as these but the results are in agreement with the idea that the shunt pathway is of minor importance to the glycolyzing red cell under normal conditions. Murphy[9] has noted that the Embden-Meyerhof pathway operates well at normal *p*H of blood and that the pentose shunt pathway becomes significant only as the *p*H drops into the more acid regions. Bartlett and Marlow[1]

TABLE 2. *Compounds Having No Effect on the Nucleotide Pattern of Incubated Human Blood*

Compound	Concentration
Methylene Blue	1.3×10^{-6}M
Thiamine	1.5×10^{-3}M
Pyrithiamine	1.2×10^{-3}M
Oxythiamine	1.7×10^{-3}M
Nicotinamide	4.4×10^{-3}M
DBI (diphenylbiguanide)	2.4×10^{-3}M
Chlorpromazine	1.5×10^{-4}M

had already estimated that the shunt pathway contributed little of the total energy under physiological conditions.

The lack of effect of nicotinamide is perhaps not surprising under the conditions utilized in these experiments, although it is known that in hemolysates, DPN disappears quite rapidly[7] and that added nicotinamide can help to deter this effect.

The oral hypoglycemic agent, DBI (diphenylbiguanide), was tried in this glycolyzing system to see if, through some action on glucose utilization, it might affect the blood nucleotide pattern. Its failure to have an effect adds no information to its poorly established mode of action.

Chlorpromazine is structurally related to methylene blue and again, it has no demonstrable effect on the blood nucleotide pattern, *in vitro*.

From the above experiments, one is led to the conclusion that interference with the Embden-Meyerhof cycle will markedly affect the blood nucleotide pattern. This is clearly shown by adding fluoride, iodoacetate, arsenate, or 2-deoxyglucose. The marked difference between the action of iodoacetate and p-chloromercuribenzoate suggests that a sulfhydryl inhibitor *per se* is not sufficient, but differences in penetration may explain the findings. The pentose shunt pathway would seem to be of little importance in the blood at a normal *p*H since neither methylene blue or thiamine enhance the nucleotide pattern nor thiamine antagonists interfere with it. This does not imply that the shunt pathway might not be of major importance in stored blood as the *p*H falls due to lactic acid accumulation. The possible effects of uncoupling agents in this system need additional confirmation before mechanisms should be discussed.

Acknowledgments

The author wishes to express his appreciation to Mr. David M. Rankine for his technical assistance and to Dr. John H. Talbott for his encouragement in these studies.

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Studies on Serum and Infectious Hepatitis Viruses of Man*

I. Preliminary Report on the Isolation of Serum and Infectious Hepatitis Viruses from Man in Tissue Culture

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A preliminary report of the results so far describe briefly the recovery of the serum hepatitis virus from five human volunteers who acquired serum hepatitis after inoculation with prototype B SH virus and from one patient acutely ill with (presumably transfusion caused) serum hepatitis, as well as the recovery of IH virus from two patients acutely ill with infectious hepatitis. In all instances the SH and IH viruses have been passaged six to 13 times in stable lung cell tissue culture with a different but characteristic cytopathologic effect for the SH and IH viruses. Titration of the SH viruses showed that the $TCID_{50}$ per ml. ranged from $10^{-4.5}$ to $10^{-6.0}$ per ml. and the IH from $10^{-5.5}$ to $10^{-6.6}$. Neutralizing antibodies against two SH viruses were produced in rabbits and against one IH virus in the guinea pig. A definite serological relationship has been established by neutralization tests between the virus from one of the SH volunteers and the virus from the patient with SH; between the two IH viruses; and between the SH and the IH viruses. The identity of the SH virus isolate from one of the volunteers was demonstrated by the production of neutralizing antibodies in a rabbit inoculated with NIH plasma pool #6, although attempts to grow virus from the plasma pool failed. The SH viruses are not inactivated by heating at 60 C. for four to six hours, the IH viruses are. SH virus killed mice and was recovered in tissue culture from their livers. IH virus did not infect mice. Both viruses have been grown also in trypsinized rabbit, guinea pig and hamster kidney tissue cultures and in human embryonic tissue cultures. Neither virus will grow in monkey kidney, ruling out most other enteric and adenoviruses of human origin. Further reports will be made as the studies continue.

IN 1956, Rightsel, *et al.*,[5] reported the cultivation of cytopathogenic agents in tissue culture from patients with clinical viral hepatitis. More recently, McLean[2] described studies involving the isolation of 43 agents from materials obtained from humans with viral hepatitis. The purpose of this preliminary report is to record the results obtained thus far from: (1) The inoculation of human volunteers with the serum hepatitis virus, its recovery in tissue culture from their blood, and the subsequent development of a specific antibody for the serum hepatitis virus both in animals and in the blood of these individuals; (2) the recovery of infectious hepatitis viruses in tissue culture from clinically ill patients, their serologic relationship, and the development of specific animal antibodies; and, (3) the serologic relationship of serum and infectious hepatitis.

Infection of human volunteers with serum hepatitis virus: Serum hepatitis virus (SH) prototype B, (icterogenic plasma pool #6[4]) was received from Dr. Roderick Murray, National Institutes of Health, in the form of lyophilized plasma. This material was reconstituted in distilled water and served as the inoculum for tissue cultures and for five human volunteers at the Arizona State Prison.

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Each of the five men was injected intravenously with 1 ml. of the SH virus containing plasma and all of them developed acute or subacute viral hepatitis. Pre-inoculation serum was obtained from each of them. Thereafter, blood was collected once a week for five months and every 30-60 days thereafter for a study of the persistence of the viremia and the possibility of the subsequent development of specific antibody. During the incubation period of the disease, all five men developed a prodromal illness characterized by moderate to severe weight loss, anorexia, vomiting, nausea, and lassitude, followed by jaundice. Two of the five men had dark brown urine. Three of the five ran a low grade fever (38 to 39 C.) and complained of right upper quadrant abdominal pains. Table 1 shows the details of the beginning, duration and the end of the icteric phase of the illness and the serum transaminase levels found, clearly demonstrating that these five men developed serum hepatitis as a result of the inoculation. All of them recovered from the experimental serum hepatitis.

Clinically diagnosed cases of serum and infectious hepatitis: Serum was received from one case (NB) of clinically diagnosed homologous serum hepatitis, not unlike that occurring in the more seriously ill of the five human volunteers infected with SH virus. Four months prior to the onset of jaundice, this patient had received 11 blood transfusions. Materials were also received from two hospitalized cases (HOV and MJ) of clinically diagnosed infectious hepatitis (IH). Of the two cases, one was a fatality (MJ) and the final diagnosis was "viral hepatitis," but there was no history of blood transfusion.

Isolation of SH and IH virus in stable human cell cultures: The SH serums obtained from the five volunteers during the incubation period of the disease and from the patient NB during the icteric phase of his serum hepatitis served as the inoculum for tissue cultures. Stool specimens from both cases of infectious hepatitis were prepared as 20 per cent suspensions, centrifuged at 12,000 r.p.m. for ten minutes in a refrigerated centrifuge and liver tissue, obtained from the fatal case of infectious

TABLE 1. *Serum Transaminase Levels and Icteric Periods for Human Volunteers Injected with SH Virus and Patients with IH Virus from which Viruses were Isolated in Tissue Culture*

	Patient No.	Icteric phase*			Serum transaminase levels**				Character of disease
					Highest		Lowest		
		Start	End	Duration	GOT	GPT	GOT	GPT	
SH	217	91	162	71	980	2500	88	70	Severe
	193	105	119	14	430	700	130	210	Severe
	211***	70	71	1	57	16	57	16	Mild
	210	131	162	31	700	680	320	420	Mild
	204	91	98	7	1020	1500	210	390	Mild
	NB	J		10	1360	1380			Severe
IH	HOV	J		10	>500	>500			Mild
	MJ	J		7	900	1000			Fatal

* Day Post Inoculation of SH Virus.
** Highest and Lowest Serum Transaminase Levels during Iceteric Phase of Illness.
*** Volunteer 211 had Transaminase Values of 500 and 240 fourteen days prior to Jaundice.
J = Jaundiced on hospital admission with diagnosis of hepatitis.
Blank Spaces — No data.

TABLE 2. *Summary of Results for IH and SH Virus in Tissue Cultures*

Virus Code	Virus Source	Stable lung	TC passage level: Primary culture* BR	Kid	Lu	Sk	RK	GPK	HK	MK	Mouse inoc.	$TCID_{50}$ ml.
193	Serum	11					+	+	+	−	0/6	$10^{4.5}$
210	Serum	13					+	+	+	−	0/6	$10^{5.5}$
217	Serum	10					+	+	+	−	0/8	$10^{4.5}$
211	Serum	10	+	+	+	+	+	+	+	−	2/5	$10^{6.0}$
204	Serum	10					+	+	+	−	5/5	$10^{4.5}$
NB	Serum	6					+	+	+	−		$10^{5.5}$
204M	Liver	2										
HOV	Stool	13	+	+	+	+	+	+	+	−	0/6	$10^{5.5}$
MJ	Liver	8	+	+	+	+	+	+	+	−	0/6	$10^{6.5}$

* Primary tissue cultures: BR—Human Brain, Kid—Kidney, Lu—Lung, Sk—Skin, RK—Rabbit Kidney, GPK—Guinea Pig Kidney, HK—Hamster Kidney, MK—Monkey Kidney.
+ = 2/2 cultures showed 4+ CPE after inoculation of 10,000-1000 $TCID_{50}$ Virus.
204M = Mouse Liver; MJ Virus = Human Liver.
Blank Spaces = Not done.

hepatitis, was treated in a similar manner. Supernatant fluid from these materials served as the inoculum for tissue cultures. Tissue culture cells were prepared from a stable line of human lung cells[1] grown in Medium No. 199[3] with 20 per cent calf serum. Materials were inoculated into replicates of tissue cultures. Each tube received 0.2 ml. of the desired material which was permitted to absorb for 30 minutes at room temperature. One ml. of maintenance medium (Medium No. 199 with 2 per cent calf serum) was then added to each tube. These cultures were incubated at 36 C. until cytopathogenic effects (CPE) had occurred, or for seven days, whichever came first. Virus was grown from all specimens except the stool from the fatal case (MJ) The tissue culture fluids were frozen and thawed two times between each virus passage. Employing this procedure, both the SH and the IH viruses were passaged six to 13 times in tissue cultures systems with a characteristic CPE. The results are summarized in Table 2.

Neutralization of SH and IH viruses in stable cell cultures: Serum obtained from volunteer #210 (8/26/60) was employed in two ways. In the first instance, it was passaged 13 times in tissue culture. After high speed centrifugation, tissue culture SH virus antigen (#210-TC13) was used for rabbit immunization to produce an antiserum against this tissue culture SH virus. In the second, the original #210 SH serum, without tissue culture passage, was employed as the virus antigen for the production of antiserum against this SH virus in a rabbit. Antiserum #210 was employed against the NB virus and antiserum #210-TC13 was used against #210-TC13 virus in neutralization tests. Antiserum against the MJ virus was produced in a guinea pig and was employed in neutralization tests against the homologus virus. The results are shown in Table 3. These clearly show that antiserum against SH #210-TC13 virus neutralized 100 to 1000 $TCID_{50}$* of this virus through the fourth day. In the case of the #NB-TC-3 virus, a

* $TCID_{50}$ means "Tissue Culture Infecting Dose." The figure 50 indicates that this is the amount of TC virus which will result in virus cytopathology in 50 per cent of the cultures inoculated.

distinct neutralization of at least 10,000 $TCID_{50}$ was noted through the seventh day, thus establishing the serological relationship of #NB-TC-3 to the original source of the #210 tissue culture virus, the blood of volunteer #210. The original icterogenic plasma pool #6 from the National Institutes of Health was also employed for animal inoculation. One rabbit was given 10 ml. of icterogenic plasma intravenously and 5 ml. intramuscularly once a week for three weeks. One week after the last injection, the rabbit was bled and a 1:20 dilution of the serum was used in neutralization tests against the SH virus isolate #210. This rabbit antiserum neutralized 100-320 $TCID_{50}$ of SH virus #210, thereby establishing a definite serologic relationship with the SH agent in the icterogenic plasma pool #6 with that recovered from volunteer #210. Although more than 100 attempts were made under several different conditions, virus was never recovered in tissue culture from pool #6.

Heat Treatment of SH and IH virus: The six SH viruses are not inactivated by heating at 60 C. for four hours and one has been heat treated for six hours with virus survival. In fact, the CPE is enhanced as though the virus is freed from some inhibitor. In sharp contrast, the HOV IH virus was inactivated when exposed to 60 C. for one hour. However, the MJ IH virus was somewhat more stable.

SH and IH viruses in mice: Five SH and two infectious hepatitis viruses were each inoculated into a group of newborn mice (0 to 48 hours old). The results are shown in Column 6 of Table 2. Five mice inoculated with SH virus #204 were found moribund on the fifteenth day of infection. The livers of two of these mice were removed, prepared as 10 per cent suspension in M-199 with 2 per cent calf serum, centrifuged at 12,000 r.p.m. for ten minutes, and inoculated into two stable lung cell tissue cultures. Within 72 hours both inoculated cultures showed a characteristic CPE, while two control (uninoculated)

TABLE 3. *Neutralization of SH and IH Viruses with Specific Antisera Produced in Animals*

		SH virus						IH virus		
	Virus dilution	210 – 13*			NB – 3*			MJ – 3*		
		4d	6d	7d	4d	6d	7d	4d	6d	7d
Virus Titration	1:10	++	++	++	++	++	++	0 0	++	++
	1:100	++	++	++	++	++	++	0 0	++	++
	1:1000	++	++	++	0 0	++	++	0 0	++	++
	1:10,000	0 0	++	++	0 0	0 0	++	0 0	++	++
Serum Virus Mixture	1:10	0 0	++	++	0 0	0 0	0 0	0 0	++	++
	1:100	0 0	0 0	++	0 0	0 0	0 0	0 0	0 0	++
	1:1000	0 0	0 0	++	0 0	0 0	0 0	0 0	0 0	++
	1:10,000	0 0	0 0	++	0 0	0 0	0 0	0 0	0 0	++
	Serum Controls	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

++ = 4+CPE on day indicated for each of two tissue cultures.
d = days
* = 210-TC13 = passage level of SH virus in tissue culture against homologous antiserum produced in rabbit. NB-3 = NB virus against antiserum from blood of rabbit inoculated with 210 serum from which 210-TC13 virus was isolated. MJ-3 = MJ virus at 3rd tissue culture passage level in tissue culture against homologous antiserum. Titer of virus used in Neutralization Test was $10^{-4.5}$, $10^{-5.5}$, $10^{-5.0}$, respectively.

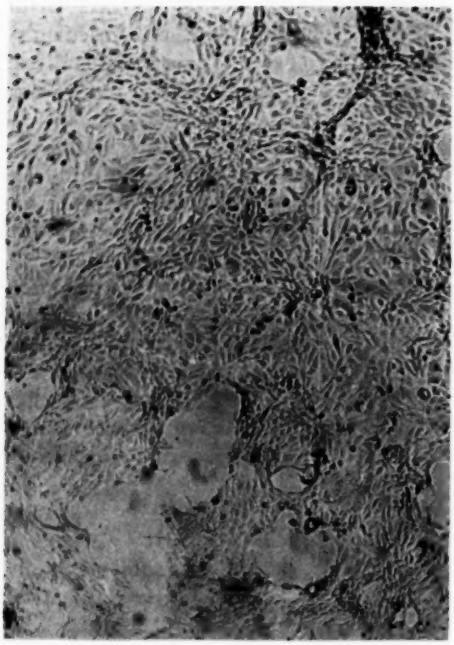

FIG. 1. CONTROL CULTURE—Control uninfected rabbit kidney tissue culture at five days.

cultures remained normal. A second passage was made with the same results. None of the mice inoculated with IH viruses showed evidence of infection during 14 days of observation.

SH and IH virus in primary cell cultures: Both the SH and IH viruses were passaged twice in trypsinized monkey kidney tissue cultures. Inoculum for the initial passage for each virus was at least 10,000 $TCID_{50}$ virus. In no instance was cytopathology observed during two blind (control) passages. Passage of both SH and IH virus into primary human embryonic tissue cultures resulted in CPE in three to five days after inoculation. Second passage skin and brain tissue cell cultures failed to show characteristic CPE when inoculated with first passage virus from the human embryonic tissue culture. However, both second passage lung and kidney tissue cell cultures showed a four plus CPE five days after inoculation with first passage viruses. Inoculum for these cultures was a 1000 to 10,000 $TCID_{50}$ dose. The SH and IH viruses listed in Column 1 of Table 2 were each passaged three to five times in primary tissue cultures of trypsinized rabbit, guinea pig and hamster kidney. Five to seven days after inoculation all cultures showed a characteristic four plus CPE. SH and IH viruses at the fifth passage level in rabbit kidney cultures (RKC) titered $10^{-5.5}$ to $10^{-7.0}$. Maintenance medium for RKC was M-199 with 3 to 6 per cent normal rabbit serum.

Neutralization test in rabbit kidney cultures: Tests were performed with RKC grown SH and IH viruses, convalescent serum from the volunteers, normal human sera and specific antisera produced in rabbits against both SH and IH viruses. These sera were tested against increasing dilutions of the desired virus. Rabbit antisera and volunteer convalescent serum were used at a final dilution of 1:40, while normal human serum was used at a 1:10 final dilution. SH and IH rabbit antisera neutralized at least 10,000 and 1000 $TCID_{50}$ of homologous virus, respectively. Volunteer convalescent serum neutralized 1000 $TCID_{50}$ SH virus #211. Normal human serum did not neutralize the SH or IH viruses. All of the five SH volunteer convalescent sera have shown specific antibody against the SH virus.

Neutralization tests were performed using rabbit SH #210 antiserum against HOV and MJ (IH) viruses. Results showed that SH antiserum #210 neutralized 1000 $TCID_{50}$ MJ (IH) virus and 100 $TCID_{50}$ HOV (IH) virus establishing a serological relationship between SH virus #210 and the IH viruses, MJ and HOV. Gross neutralization tests were performed using MJ and HOV viruses against MJ and HOV rabbit antisera. Each antiserum neutralized 100 $TCID_{50}$ of each heterologous IH virus demonstrating a re-

lationship between the 2 IH agents. The appearance of the normal rabbit kidney culture is shown in Figure 1. Figure 2 shows the typical CPE at five days after inoculation with SH virus and Figure 3 shows the protective effect of the animal antibodies in a tissue culture five days after inoculation with a virus-serum (antibody) mixture.

Discussion

It was believed important to make this brief preliminary report of our early results, since the work at this point indicates success in isolating and growing virus agents from patients with serum and infectious hepatitis, the development of specific neutralizing antibodies in animals, and definite serologic relationships between the SH isolates, the IH isolates, and the SH and IH isolates. The identity of the SH virus has been demonstrated both by the development of specific neutralizing antibodies in the human volunteers and in animals inoculated with the original source, icterogenic plasma pool #6 (NIH). The failure of the viruses to cause cytopathology in monkey kidney tissue culture indicates that we are not dealing with any of the three polio viruses; Echo viruses, types 1 through 28; Coxackie viruses, group A, types 1 through 23, or group B, types 1 through 6; or the adenoviruses, types 1 through 18. These studies will be reported in greater detail subsequently.

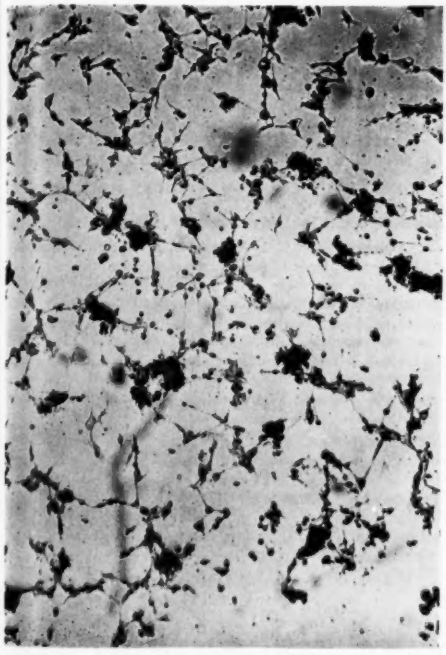

FIG. 2. VIRUS CYTOPATHOLOGY – Five days after inoculation with 1000 $TCID_{50}$ SH virus (211).

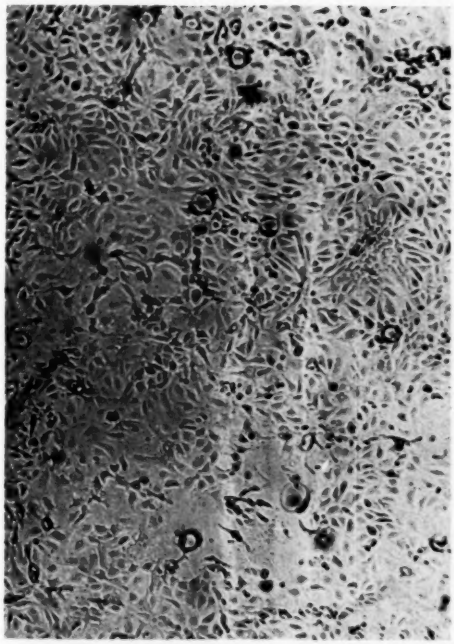

FIG. 3. VIRUS NEUTRALIZATION BY SPECIFIC ANTIBODIES—Five days after inoculation with volunteer serum 211 mixed with 1000 $TCID_{50}$ SH virus (211).

Acknowledgment

We wish to express our sincere appreciation to the Superintendent of Arizona State Prison, Mr. Frank A. Eyman, and Dr. Elmer Heap, the prison physician, for their valuable assistance and to the five volunteers who so courageously cooperated to make it possible for these studies to be done with known SH virus.

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STATEMENT OF THE OWNERSHIP, MANAGEMENT AND CIRCULATION REQUIRED BY THE ACT OF CONGRESS OF AUGUST 24, 1912, AS AMENDED BY THE ACTS OF MARCH 3, 1933, JULY 2, 1946, AND JUNE 11, 1960 (74 STAT. 208)

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(Signed) GEORGE F. STICKLEY

Sworn to and subscribed before me this 11th day of September 1961.

KATHARINE K. STOUT
Notary Public

(Seal)

My commission expires on February 2nd, 1965.

Magnetoelectrophoresis*

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J. WALTER KELLER, JR.†
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The theory of magnetoelectrophoresis of erythrocytes is described and a system of red cell separation from plasma is presented in detail. The apparatus has been designed and built and tested to demonstrate its effectiveness. The steps necessary to make this process applicable to the routine donor room are described.

The purpose of this investigation was to develop and perfect a process for the selective removal from the body of either plasma or the cellular elements of the blood, returning the unneeded components undamaged to the donor.

Certain basic requirements must be satisfied to make a method suitable for the application proposed. First, it must be reliable and simple so that it can be used in the average blood bank without complex equipment or unusual skills. The use of a closed system is necessary, and all parts contacting the blood must be of plastic or other inert materials constructed in an economical fashion so that they are disposable. While separation is to be effected outside the body, the mechanism accomplishing it will not be detached from the donor until collection and return are completed.

THE SEPARATION of cells from plasma can be accomplished at present by means of the standard centrifuge or by the continuous centrifuge developed by Cohn.[9] the former method involves excessive time as well as separation of the blood from the donor during centrifugation. The collection of a full unit (500 ml.) of either cells or plasma requires two separate operations and at one time the donor may be deficient to the extent of 750 ml. of fluid. Once blood is separated from the donor, an adequate cross match is necessary to assure identity and safe return. The Cohn retention bowl type of centrifuge separates continuously during collection but is costly and complex to operate and does not lend itself to economical mass collection. It is, therefore, apparent that new and better methods are needed to achieve the desired goal.

A method, arising from the results of Kolin and co-workers working with static systems (1-8), has been found wherein the combined forces supplied by the passage of an electrical current through the blood in the presence of a high magnetic field causes movement of cells relative to plasma in a flowing system. This force can be applied from outside of a plastic tube or through its wall without opening the system with the attendant risk of contamination. The system provides the possibility of collecting blood from the donor and passing it through a magnetic field where it is acted upon by an electric current, causing separation and passage of cells out through one tube and plasma out through another as they leave the magnetic field. Selectively, the contents of either tube may be returned to the donor while collection from the other is continued. This system may also be used for the separation of cells from

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TABLE 1

Material	Time of centrifugation (min.)	Frequency	Temp.	10^3K (mho cm^{-1})
ACD Blood	45	60	0	
P				7.7
C				1.33
30ml ACD Blood plus 6 ml ACD	45	60	0	
P				6.6
C				1.07
30ml ACD Blood plus 6 ml H_2O (0.125 M NaCl Some Hemolysis)				
P				5.75
C				1.23
30 ml ACD Blood plus 0.3 ml 5M NaCl (0.20 M NaCl)				
P				9.3
C				2.2
ACD Blood	60	60	0	
P				6.74
C				0.67
ACD Blood	60	60	25	
P				12.8
C				1.33
ACD Blood	60	60	37	
P				15.9
C				1.71
ACD Blood Plasma & cells warmed to 37 C. & returned to zero	60	60	0	
P				6.60
C				0.70
ACD Blood	60	0	0	
P				6.6
C				0.72
ACD Blood	60	0	37	
P				16.1
C				1.8

other fluids, such as a special preservative or anticoagulant before a unit of stored blood is transfused or otherwise processed.

Kolin's work has shown that the well-known force on a conductor in a magnetic field gives rise to the migration of particles suspended in a fluid if the two phases differ in electrical conductivity. This migration has been termed magnetoelectrophoresis. The force exerted is in fact similar to that force exerted on the rotor of an electric motor when current is flowing in the stator.

In essence, the movement of cells in the

plasma is dependent upon a difference in conduction between the cellular elements and the fluid elements of blood. This difference is illustrated in Figure 1a wherein a sphere or cell of high conductance distorts the current density to increase it inside the cell. In Figure 1b, a sphere of low conductance decreases current density as shown graphically. One can think of a particle as being displaced by the liquid in much the same way that particles of low density are made to rise during sedimentation. It can be shown theoretically that the velocity of migration of spherical particles is proportional to the square of the radius; that is, large particles migrate more rapidly than small particles. From theory, additional predictions can be made which are of great practical importance in the design and operation of magnetoelectrophoretic equipment. One of the most important of these concerns the effect of irregularities or inhomogeneities in electric current density or magnetic field. Such regions, *e.g.* at the edges of the magnetic field, may cause electromagnetically engendered convection in the fluid and thus nullify separation. These factors have been duly considered in the design and construction of our magnet pole pieces and the plastic separation cell.

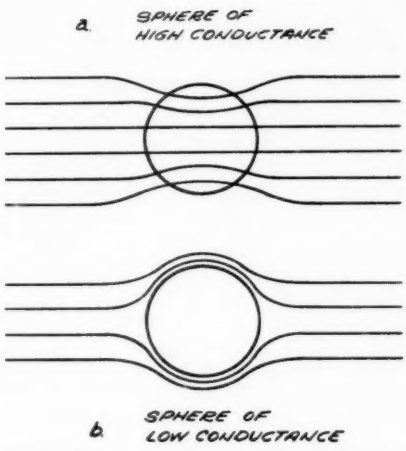

FIG. 1. Effect on current density of high and low conductance particles.

As noted, the size, shape and conductance of the particle and medium are of major importance in determining the magnetoelectrophoretic behavior. The results of conductance measurements made on plasma and cells packed by centrifugation at 500 times gravity for forty-five minutes to one hour are presented in Table 1. The con-

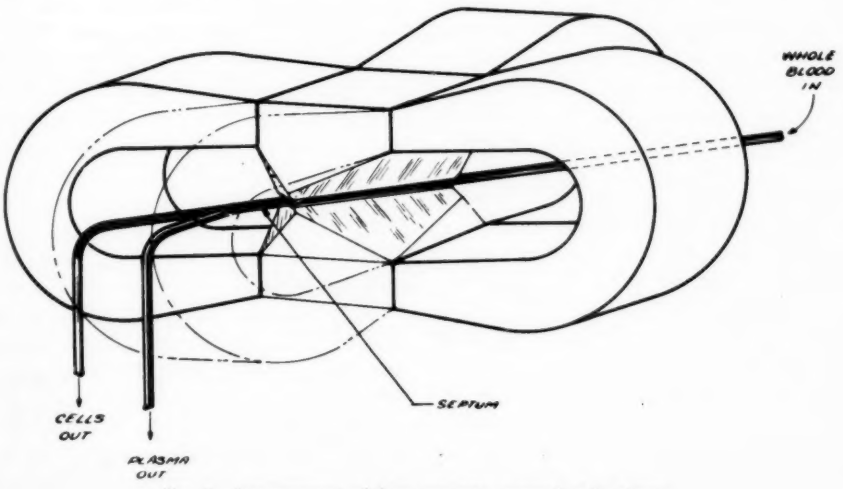

FIG. 2. Arrangement of four magnets around pole pieces.

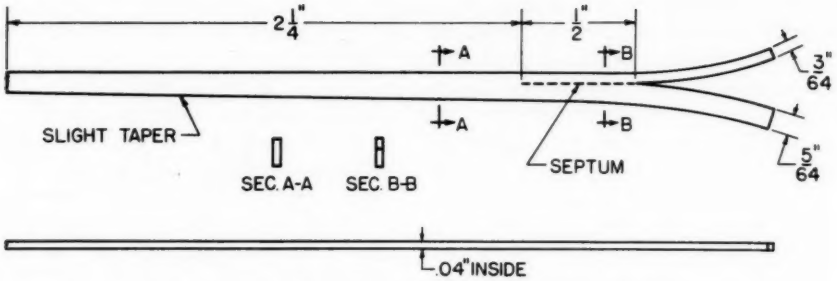

FIG. 3. Detail of separation cell. Plastic separation chamber.

ductance of erythrocytes (C) is quite small compared to that of plasma (P), probably less than one-tenth, and the difference persists at elevated or lowered salt concentrations and at all temperatures between 0 and 37 C. There is no important effect of frequency up to 60 cycles. The conclusion is that the conductance factor is very favorable for differential migration of cells and plasma without critical control of salt concentration.

The shape of red cells as it relates to the local effect on the current density vector, deserves some comment. A simple model which should have most of the geometrical characteristics of erythrocytes would be a short cylinder or flat disc. Accordingly, circular discs about .020 inch in diameter and .001 inch thick were cut from plastic sheeting and their behavior observed while lying horizontally in a fine glass tube filled with 0.15 M sodium chloride. The solution had a current density of about 0.25 amp cm^{-2} and was situated in a magnetic field of about 23 kgauss. Cylinders cut from Mylar proved to be para- or ferromagnetic so that with no current, the rest position was with the axis of the cylinder and that of the glass tube parallel. Polystyrene discs with no current passing lay at rest on the bottom of the tube with the disc axis vertical.

With both Mylar and polystyrene discs, rapid migration in the direction perpendicular to the current and magnetic field occurred when the current was started. It was concluded that the shape of the red cells should in no way prevent their movement in the electromagnetic field.

Since the fields attainable with electromagnets are more intense than those with permanent magnets, the former would be preferable for magnetoelectrophoretic purposes. The velocity of migration is

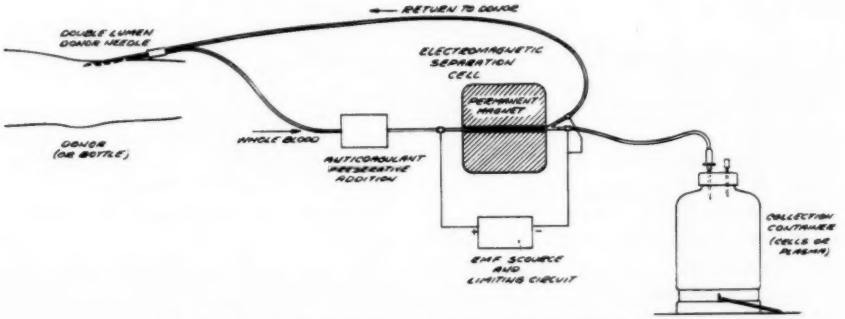

FIG. 4. Complete magnetoelectrophoresis separation system.

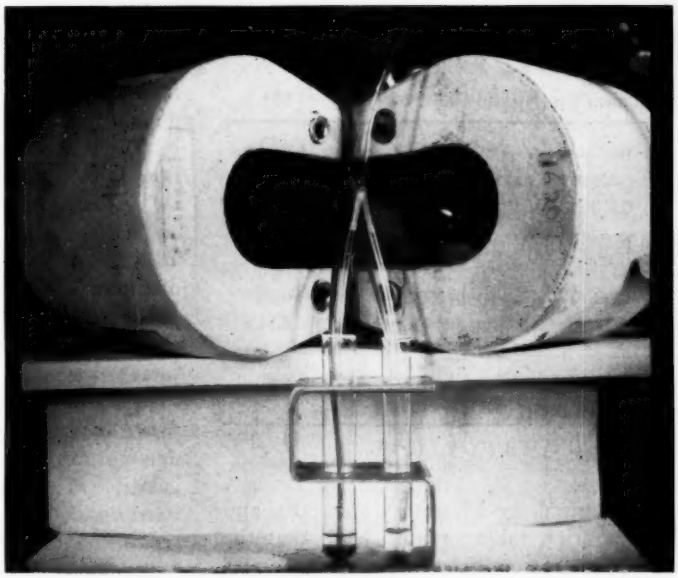

FIG. 5. Laboratory separation system showing cells on left and plasma on right.

found to be just proportional to the magnetic field intensity; therefore, the greater the magnetic field available, the better the separation. However, for reasons of simplicity and economy, permanent magnets are much to be preferred since a small electromagnet could consume five to ten kilowatts of power. This would make use of this type of apparatus difficult in the routine donor room because of the lack of availability of power. We have investigated various ways of combining the fields of a number of large permanent magnets with the appropriate pole pieces and have attained fields of as high as 22 kgauss in a space of 2.5 inches long by one-half inch wide by .060 inch deep (Fig. 2). Measurements of field intensity were made, using a Dyna-Empire gaussmeter calibrated either with a one kgauss standard magnet or with an air core solenoid of known geometry giving a field of 330 gauss. The methods check to within about 1 per cent. It was, however, assumed that the linearity curve of the gaussmeter probe as supplied by the manufacturer was correct.

The distance traversed by a particle under the influence of the electromagnetic force is proportional to the product of the current density, the magnetic field strength and the time within the field. Thus, it is advantageous to have the cell long and narrow so that the particle is within the field as long as possible but does not have to migrate a great distance. Because of the electromagnetically engendered convection which occurs at the edges of the field, it is necessary to complete any separation within the field and remove the fractions through separate tubes. To accomplish this end, we have employed a molded plastic tube of rectangular cross section with a septum near one end which serves to prevent remixing of the fractions as the tube exits from the field (Fig. 3).

The current density is limited by the heating effect produced which, if uncontrolled, causes thermal convection and the liberation of oxygen or other dissolved gases from the cells or plasma.

The complete system is shown diagrammatically in Figure 4, while in Figure

TABLE 2. *Effect of Glycerol Pectate, Dextran and Cell Concentration*

Initial cell concentration	Additive	SF*
0.1	2% GP	0.43
0.25	2% GP	0.48
0.5	2% GP	0.50
0.25	2% GP	0.48
0.25	1% GP	0.32
0.25	0% GP	0.16
0.25	2% Dextran	0.14
0.5	2% GP	0.50
0.5	0% GP	0.23

* $SF = (1\text{-}C_p/C_c)\ (V_p/V_t)\ (1/1\text{-}C_t)$

5 is pictured the laboratory apparatus and a separation taking place. Note that the red cells are coming out the left tube and the plasma is coming out the righthand tube.

In order to measure quantitatively the degree of separation accomplished, it is expedient to define a "separation factor," S.F. Let C be the concentration (volume fraction) of cells in the "cell" and "plasma" fractions and in the initial cell suspension denoted by the subscripts c, p and t respectively. Let V be the corresponding volumes.

$$SF = (1\text{-}C_p/C_c)\ (V_p/V_t)\ (1/1\text{-}C_t)$$

Other factors being constant, this factor increases whenever (a) the hematocrit of the cell fraction increases, (b) a larger fraction of the total volume is removed as plasma or (c) when the hematocrit of the initial cell suspension is increased. The range of SF is from zero (no separation) to unity (complete separation). For a cell suspension with an initial hematocrit of 45 ($C_t = 0.45$) an SF of one would be obtained when $C_p = 0$ (no cells in the plasma fraction) and when the volume of the plasma fraction was 55 per cent of the total volume.

In the following experiments, the magnetic field was 22 kgauss, the current density was 0.25 amp cm^{-2} and the linear velocity of fluid flow was about 50 cm min^{-1} with the total distance available for separation being 5 cm.

For whole blood, the migration is very slow with the value of SF about 0.2. Upon diluting blood with plasma about two-fold, the separation factor showed only minor changes.

Substantial increases in SF can be obtained by agents which cause the cells to clump together. This effect is readily understandable when particle size is considered. The clumped cells behave as larger particles and thus migrate more rapidly. Thus a cell suspension containing cells at a volume fraction of 0.5 (whole

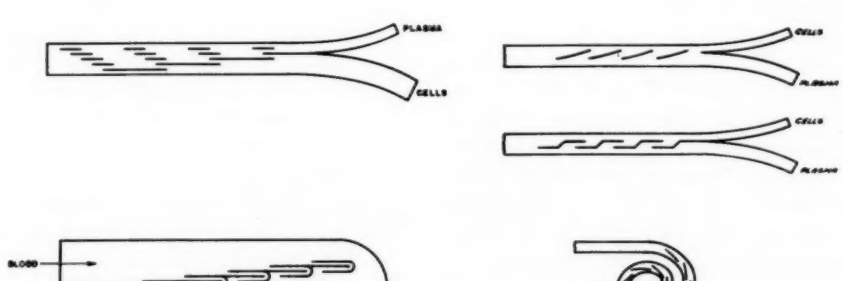

FIG. 6. Multistage cells. Proposed multistage separation chambers.

blood) and having 2 per cent glycerol pectate added gives SF about 0.5. A similar effect is obtainable by diluting the cells in pooled plasma (presence of isoagglutinins). Results are shown in Table 2.

The aggregating effects of glycerol pectate can be reversed by dilution of the erythrocyte suspension in plasma. At 1 per cent glycerol pectate, strong clumping is obtained (microscopic examination). Upon dilution of the clumped suspension in plasma, some clumps were observed at 0.25 per cent glycerol pectate but practically none were present at 0.1 per cent.

The plasma fraction from a separation containing glycerol pectate can be recycled with a second enrichment resulting. This fact suggests that multistage cells should result in satisfactory separations even with low values of SF.

The method of separation presented here appears to have value in many phases of blood collection, handling and transfusion. Magnetoelectrophoresis is described and a laboratory apparatus to accomplish it has been built. There are still problems which must be resolved before this can become a technic in routine use. The separation factor of cells from plasma, after one passage through a separating cell of maximum magnetic field density and moderate current, is low and probably inadequate for routine mass blood collection. Increased current density will improve the separation factor, but as the current is raised, an undesirable amount of heat is produced. While a refrigeration system could be added to maintain normal temperature, it should be avoided, if possible, as a clumsy and undesirable complication. However, the simple step of passing the separated cells and plasma through a second separation chamber in the same magnetic field produces an approximately equivalent separation which may be added to the original. It is, therefore, reasonable to assume that were a number of cells arranged in series and parallel, they might accomplish rapid and complete separation suitable for clinical use. Although the present magnet pole pieces are saturated with a maximum flux density, the magnets used are capable of saturating a much larger pole piece. This will be necessary in using complex chambers such as those suggested in Figure 6.

While one can design such a cell theoretically, it must be built and tested before all of the potential problems can be solved with certainty. Among the most complex problems are turbulence and flow rates in any chambers so complicated. In addition, since it is necessary to pass a current through the solution, uniform current densities may be difficult to establish. While chambers designed to resolve these problems may appear to be complex in shape, they may be readily produced by plastic molding processes.

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List of Plants Tested for Hemagglutinating Activity*

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Lists are given of all the plants tested in the authors' laboratory for hemagglutinating activity. The plants, arranged alphabetically by their scientific names, are grouped into three tables: (1) plants found to contain blood group specific lectins, (2) plants which gave negative or nonspecific extracts, and (3) plants which yielded hemolytic extracts.

The importance of negative findings should not be overlooked. It is frequently difficult to convince investigators of the need for publishing what they may consider fruitless work. Documentation of such data is valuable even if the only result is to save others from repeating the same experiments. With the assistance of Dr. Mourant, who informed me of the extent of Dr. Boyd's search, we were able to convince the authors of the need for this publication. We hope that others who have unpublished negative data which may be of value to investigators will be encouraged to submit them for consideration. (Ed.)

THE IMPORTANCE of blood group specific plant agglutinins (lectins) in blood grouping work is now sufficiently recognized that we are receiving repeated inquiries as to whether or not we have tested a particular species. We therefore offer the following lists, as complete as we could make them, of plants tested in this laboratory. These include the plants published in three previous publications (Boyd and Reguera 1949, Boyd 1950, and Schertz, Boyd, Jurgelsky, Jr., and Cabanillas 1960), and a good many not previously published. Negative as well as positive results are included and in Table 3 we give a list of plants which yielded hemolytic extracts. The plants in each table are arranged alphabetically by their scientific names.

The samples (seeds or dried mushroom fruiting body) were ground fine in a mill and extracted overnight with ten times their weight of a 0.9 per cent NaCl solution. The extracts were tested as described in a previous paper (Boyd and Reguera, 1949).

Table 1 contains the names of plants found by us to contain blood group specific agglutinating activity. The name of the plant is followed by the observed specificity and the reference to the publication by the original discoverers, when this is known to us.

Table 2 is a list of plants found by us to be negative or nonspecific for human erythrocytes. These plants have been tested at various times and with varying degrees of completeness; the level of testing is indicated by a symbol following the name of the plant. (See symbols, p. 375). Common names have been given as well as the scientific names when it seemed possible that they could be an additional aid in identifying the particular species or variety used.

Examination of Table 2 will show that the great majority of the plant extracts tested by us have been either inactive or nonspecific. However, we would never have continued this work except for the fortunate accident that the first plant tested, the seeds of *Phaseolus lunatus*, proved to be specific for blood group antigen A. Since then we and others have found other specific extracts. Those tested by us are listed in Table 1.

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TABLE 1. *Plants tested by us and found to contain blood group specific lectins.*

*Alysicarpus** *vaginalis* (B) (Schertz, Boyd, Jurgelsky and Cabanillas 1960). *Arachis hypogaea* ("Gy") (Boyd *et al.* 1959). *Aristolochia galeata* (B) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Bauhinia monandra* (O) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Bauhinia purpurea* (N) (Mäkelä 1957, Boyd, Everhart, and McMaster 1958). *Bixa orellana* (B)* (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Cochlospermum gossypium* (O) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Crotalaria breviflora* (A_1+A_2) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Crotalaria intermedia* (B)** (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Crotalaria sp.* (A_2) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Cytisus sessilifolius* (H) (Renkonen 1948). *Dolichos axillaris* (A_1). *Dolichos biflorus* (A_1) (Bird 1951). *Ehretia buxifolia* (O) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Euonymus europaeus* (H+B) (Schmidt 1954). *Helianthus annus* (O) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Iberis amara* (M) (Discoverer not known to us). *Lotus tetragonolobus* (H) (Renkonen 1948). *Musa textilis* (A) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Phaseolus lunatus* (Pole Lima) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Alpine) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Alpine Large) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Alpine Baby Lima Beans) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Bush Lima Beans) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*—Bailey Bush Lima Beans) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Bush or Dwarf Lima Beans) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Butter Bean-Seaside Braul) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Dwarf Lima Bean) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Empire State Lima Bean) (A_1A_2) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Fordhook Lima Beans) (A_1) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Henderson's Bush Lima) (A) (Boyd and Reguera 1949). *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*-Wood's Prolific) (A) (Boyd and Reguera 1949). *Phaseolus sp.* (Big 6) (A). *Phaseolus sp.* (Improved Giant Podded (A). *Pinanga kuhlii* (A_2) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Pueraria lobata* (A_2) (Schertz, Boyd, Jurgelsky, and Cabanillas 1960). *Sophora japonica* (B+A) (Krüpe and Braun 1952). *Tecoma stans* (A_1+A_2). *Ulex europaeus* (H) (Cazal and Lalaurie 1952). *Vicia cracca* (A) (Renkonen 1948). *Vicia graminea* (N) (Ottensooser and Silberschmidt 1953). *Vicia peregrina* (A_1). *Vicia villosa* (A_1). *Vitis aestivalis* (B) (Boyd and Reguera 1949).

* Later samples tested as negative and would belong in Table 2.

** Later samples tested sometimes negative, sometimes nonspecific, and would belong in Table 2.

TABLE 2. *Plant extracts found negative or nonspecific for human cells.*

Meaning of symbols:

+3, positive with A, B, and O erythrocytes; —3, negative with A, B, and O erythrocytes; +4, and —4 positive or negative with A_1, A_2, B, and O erythrocytes; +3R and —3R, positive or negative with A, B, and O erythrocytes, the O cells including D+ and D— types; +4P and —4P, positive or negative with A_1, A_2, B, and O cells of the Knickerbocker panel of bloods which included bloods positive and negative for antigens: C, C^w, D, E, V, c, e, K, k, Fy^a, Fy^b, Js, Le^a, Le^b, Jk^a, Jk^b, M, N, S, s, P, Lu^a, Lu^b; W, weak reactions, E, erratic reactions, and WE weak and erratic reactions.

Abies cephalonica —3, *Abrus precatorious* +3, *Abutilon avicennae* (Manchurian Jute) —3, *Abutilon vitifolium* —4, *Acacia armata* —4P, *Acacia auriculaeformis* —4, *Acacia baileyana* —3R, *Acacia buxifolia* —4P, *Acacia crassiuscula* —4, *Acacia cultriformis* +3R, *Acacia cyanophylla* —4, *Acacia cyclops* —4, *Acacia dealbata* (Silvergreen-wattle A) —4P, *Acaria decora* (Graceful-wattle A) +4P, *Acacia decurrens* (Green Wattle) —3R, *Acacia discolor* (Sunshine-wattle A) —4P, *Acacia elata* —4, *Acacia falcata* (Burra A) —4P, *Acacia farnesiana* (Sweet A) —4P, *Acacia giraffae* (Giraffe A) —4P, *Acacia homalophylla* (Gidgee A) —4P, *Acacia horrida* (Allthorn Acacia) +4P, *Acacia latifolia* (Broadleaf A) W, *Acacia linearis* (Needle A) —4P, *Acacia linifolia* (Flaxleaf A) —4P, *Acacia linifolia* (Prominens) —4P, *Acacia longifolia* (Sydney Golden wattle) —3R, *Acacia melanoxylon* —4P, *Acacia mollis* —4P, *Acacia obtusata* —4P, *Acacia podlalyriaefolia* —4P, *Acacia pruinosa* —4P, *Acacia pycnantha* (Golden wattle) —4P, *Acacia retinodes* —4P, *Acacia saligna* —4P, *Acacia secundiflora* —3, *Acacia spectabilis* —4P, *Acacia suma* WE, *Acacia toruosa* —4P, *Acanthorhiza warscewiczii* —3, *Acanthus mollis* —4, *Acer macrophyllum* —3, *Acer platanoides* W, *Achillea ptarmica* —3, *Aconitum lycoctonum* —3, *Acroclinium roseum* —3, *Actaea sp.* (mixed) —4, *Actinidia arguta* —4, *Actinomeris alternifolia* —4, *Actinomyces* (mould extract) —3, *Adenanthera bicolor* WE, *Adenanthera pavonina* E, *Adlumia fungosa* —4, *Adonis aestivalis* —3, *Aeschynomene americana* —4P, *Aethionema sp.* —4, *Aframomum melegueta* —4P, *Agapanthus africanus* —4, *Agaricus campestris* (Commercial mushroom) +3, *Ageratum sp.* (Blue Ball, Floss Flower) —3, *Ageratum sp.* (Blue Perfection, semi-dwarf) —4P, *Agrimonia odorata* —4, *Agrostema coronaria* —3, *Albizzia falcata* +4P, *Albizzia julibrissin* —4, *Albizzia lophantha* —4P *Alchemilla speciosa* —4, *Aleurites moluccana* —4P, *Aleurites trisperma* —3, *Algaroba sp.* W, *Alla-*

manda cathartica —4, *Allamanda neriifolia* —4P, *Allium cepa* —3, *Allium porrum* —3, *Allium sp.* —4, *Alonsoa sp.* —4, *Aloysia sp.* —4, *Alstroemeria aurantiaca* —3, *Althaea rosea* (Mixed) —4, *Althaea sp.* (Hollyhock mixed) —3, *Alysicarpus vaginalis* W, *Alyssum saxatile* —3, *Alyssum sp.* (Rock garden rare species) —3, *Alyssum sp.* —3, *Amanita muscaria* +4, *Amanita phalloides* —4, *Amaranthus caudatus* +3, *Amaranthus tricolor* (Sunrise) +3R, *Amaranthus tricolor* (Molten Fire) +3R, *Amaranthus tricolor* (Splendens) +3R, *Amberboa muricata* —4, *Amblyolepsis setigera* —4, *Amelanchier stolonifera* —4, *Ammi majus* —3, *Ammi visnaga* —3, *Amomum melegueta* —4P, *Amorpha canescens* —4P, *Amorpha fragrans nana* —4P, *Amorpha fruticosa* —3, *Amorpha nana* —4, *Ampelopsis aconitifolia* —4, *Ampelopsis brevipedunculata* —4P, *Amsonia tabernaemontana* —4, *Anagallis grandiflora* —3, *Anagallis linifolia* —3, *Anchusa barrelieri* (Blue Bird Bugloss) —3, *Anchusa myosotidiflora*, Lehm. —3, *Andropogon sorghum* (var. *saccharatum* Sweet Sudan Grass)? —4, *Andropogon sorghum* (var. *saccharatum* Sweet Sorghum)? —4, *Andropogon sorghum* (var. *sudanense* Sudan Grass)? —4, *Anemone coronaria* —3, *Anethum graveolens* —3, *Anhalonium lewinii* —3, *Annona muricata* —3, *Annona reticulata* —3, *Annona squamosa* —4P, *Anomatheca sp.* +4, *Anthemis kelwayi* (Golden Marguerite) —3, *Anthericum liliago* —4, *Antidesma bunius* —4P, *Antirrhinum majus* —3, *Antirrhinum sp.* (Rust Resistant) —4P, *Antirrhinum sp.* (Snapdragon-Red) —4, *Apium graveolens* var. *dulce* —3, *Aquilegia sp.* (Mixed) —4, *Arabis alpina* —3, *Arachis hypogaea* (Peanuts) —3, *Arachis hypogaea* (Ground Nuts-Baladi) —3, *Arachis hypogaea* (Ground Nuts-Griza bunch) —3, *Arachis hypogaea* (Ground Nuts-Griza spreading) —3, *Arctotis stoechadifolia* (African Daisy) —3, *Arecastrum romanzoffianum* —4P, *Arisaema triphyllum* —4, *Aristea (thysiflora)*? —4, *Armeria caespitosa* —4, *Armeria formosa* —3, *Armeria juniperifolia* —4, *Armeria pseud-armeria* —4, *Artabrotys uncinatus* —4P, *Artabrotys sp.* —4P, *Artocarpus communis* (Houtt) +3, *Artocarpus integrifolius* (Forst) +3, *Aruncus sylvester* —4, *Asclepias fruticosa* —4, *Asclepias sp.* (Milkweed) —4, *Asclepias tuberosa* (Butterfly Weed) —3, *Asimina triloba* —4, *Asparagus officinalis* —3, *Asparagus plumosus* —4, *Asperula orientalis* —4P, *Aster ptarmicoides* —3, *Aster sp.* (White) —4, *Aubrieta* (Rainbow Rock cress) —3, *Aucuba japonica* —4, *Averrhoa carambola* —3,. *Balanites aegyptiaca* —3, *Bandeiraea simplicifolia* E, *Baptisia australis* (False Indigo) +3, *Baptisia bracteata* +4, *Bauhinia aculeata* —4P, *Bauhinia acuminata* —4P, *Bauhinia candicans* WE, *Bauhinia cunninghamii* +4P, *Bauhinia galpinii* —4P, *Bauhinia hookeri* —4P, *Bauhinia macrostachya* W, *Bauhinia monandra* —4P, *Bauhinia pauletia* —4P, *Bauhinia purpurea* —4P, *Bauhinia reticulata* —4P, *Bauhinia retusa* —4P, *Bauhinia rosea* —4P, *Bauhinia rufescens* —4P, *Bauhinia sp.* —4P, *Bauhinia sp.* (Acra, Ghana, Africa) +4P, *Bauhinia sp.* (*3 White flowered) E, *Bauhinia tomentosa* —4P, *Bauhinia trian-*

dra —4P, *Bauhinia variegata* —4P, *Bauhinia variegata alba* —4P, *Bauhinia violacea* —4P, *Begonia sp.* —3, *Bellis perennis* (English Daisy) —3, *Bellis sp.* (Dahlborg Daisy) —4, *Bertholletia excelsa* (Brazil Nut) —3, *Beta vulgaris* —3, *Betula pendula* —4P, *Bignonia radicans* (Trumpet vine) —3, *Bletia hyacinthina* —4, *Boehmeria nivea* —4P, *Bomarea sp.* —4, *Boronia megastigma* —4, *Brachyamphus intybaceus* —4P, *Brassica alba* —3, *Brassica botrytis* (Cauliflower original) —3, *Brassica capitata* (Cabbage, Copenhagen Market) —3, *Brassica hirta* —4, *Brassica juncea* (var. *crispifolia*)? —3, *Brassica rapa* (Turnip-egyptian) —3, *Brassica rapa* (purple top white globe) —3, *Brassica sp.* (var. *botrytis*- Broccoli)? —3, *Brassica sp.* (var. *botrytis*-Cauliflower Gaizairly)? —3, *Brassica sp.* (var. *capitata*-Cabbage half-dwarf)? —3, *Bromus inermis* (Awnless Brome) —4, *Browallia americana* —3, *Browallia demissa* —3, *Browallia elata* —3, *Browallia sp.* (Sapphire Amethyst) —3, *Bruckenthalia spiculifolia* —4, *Brunnera macrophylla* —3, *Bucida buceras* +4P, *Buddleia globosa* W, *Buglossum barrelieri* —3, *Butia eriospatha* —4P, *Butia eriospatha* (Butter Daisy) —4, *Byrsonima sp.* —4P,. *Cactus sp.* (Ornamental blend) —4, *Caesalpinia armata* —4, *Caesalpinia coriacea* —4P, *Caesalpinia ferrea* —4P, *Caesalpinia mimosoides* +3, *Caesalpinia pulcherrima* —4P, *Caesalpinia sappan* —4P, *Cajanus cajan* —4P, *Cajanus indicus* —4P, *Cajanus indicus* (Mixed) —4P, *Cajanus indicus* (Red seed) —4P, *Cajanus indicus* (Variegated seed) —4P, *Calandrinia speciosa* —3, *Calandrinia umbellata* —3, *Calceolaria multiflora nana* —4, *Calendula chrysantha* —4, *Calendula sp.* (Yellow) —4, *Callicarpa americana* (American Beautyberry, D.B.) —4P, *Callicarpa dichotoma* W, *Callicarpa japonica* —4P, *Callirhoe involucrata* —3, *Callistemon citrinus* W, *Callistemon sp.* W, *Calluna vulgaris* —4, *Calochortus sp.* —4, *Calopogonium galactoides* —4P, *Calopogonium mucunoides* —4P, *Camellia japonica* —3, *Campanula medium* —3, *Campsis radicans* —4, *Cana cimarrona* —4P, *Canavalia bonariensis* —4P, *Canavalia ensiformis* (Jack bean) +3, *Canavalia ensiformis* var. *truncata* W, *Canavalia (fairosa)*? W, *Canavalia gladiata* +4P, *Canavalia maritima* W, *Canavalia (rugosperma)*? W, *Canavalia sp.* E. *Canavalia villosa* W, *Canna sp.* —4, *Capsicum grossum* (Sweet Pepper) —3, *Capsicum minimum* —3, *Caragana arborescens* (Pea-tree) —3R, *Caragana (boisi)*? +4P, *Caragana makimovicziana* +4, *Caragana pygmaea* —4, *Cardiospermum halicacabum* —4P, *Carica papaya* —4, *Carissa grandiflora* —4P, *Carthamus tinctorius* —3, *Carthamus tinctorius* (var. *inermis*-Safflower)? —4, *Carthamus tinctorius* (var. *typicus*-Safflower)? —4, *Carum carvi* (Caraway) —3, *Carum copticum* —3, *Cassia alata* —4P, *Cassia artemisioides* —3, *Cassia bonariensis* —4P, *Cassia corymbosa* —4P, *Cassia (detrimexa)*? —4P, *Cassia excelsa* —4P, *Cassia fistula* —4P, *Cassia floribunda* —4P, *Cassia fruticosa* —4P, *Cassia grandis* +4P, *Cassia javanica* —4P, *Cassia laevigata* —4P, *Cassia leschenaultiana* —4P, *Cassia marilandica* —4, *Cassia mimosoides* —4P, *Cassia multijuja* —4P, *Cassia nodosa*

—4P, *Cassia occidentalis* —4P, *Cassia patellaria* —4P, *Cassia sp.* (Rainbow) —3, *Cassia (robusta)*? —4P, *Cassia siamea* —4P, *Cassia spectabilis* —4P, *Cassia surattensis* —4P, *Cassis tomentosa* —4, *Castanea sativa* (Chestnut) —4, *Casuarina equisetifolia*, —4P, *Coffea arabica* (San Martin) —4, *Coix lacryma-jobi* —3, *Collinsia bicolor* —4P, *Colubrina (colubrina)*? —4P, *Colutea arborescens* —3, *colvillea racemosa* —4P, *Commelina coelestis* W, *Corchorus capsularis* —4, *Corchorus olitorius* —3, *Cordia glabra* —4, *Cordia obligua* —4, *Cordia sp.* (*glabra*)? —4, *Coreopsis sp.* (*leptosyne*) —4, *Coreopsis sp.* (Yellow) —4, *Coriandrum sativum* —3, *Coronilla emerus* +4, *Coronilla glauca* —4, *Coronilla varia* —4, *Corylus avellana* (Filbert) —3, *Cosmos sp.* —3, *Couroupita guianensis* —4P, *Crotalaria alata* —4P, *Crotalaria anagyroides* —4P, *Crotalaria breviflora* —4P, *Crotalaria grantiana* —4P, *Crotalaria guatemalensis* —4P, *Crotalaria incana* —4P, *Crotalaria intermedia* —4P, *Crotalaria juncea* E, *Crotalaria lanceolata* —4P, *Crotalaria maxillaris* —4P, *Crotalaria mucronata* WE, *Crotalaria mysorensis* E, *Crotalaria paulina* —4P, *Crotalaria pumila* WE, *Crotalaria retusa* —4P, *Crotalaria saltiana* —4P, *Crotalaria sericea* —4P, *Crotalaria sp.* E, *Crotalaria spectabilis* —4P, *Crotalaria striata* (brown seed) —4P, *Crotalaria striata* (green seed) WE, *Crotalaria usaramoensis* —4P, *Crotalaria vitellina* +4P, *Crotalaria zanzibarica* +4P, *Cryptostegia madagascariensis* —4P, *Cucumis melo* —3, *Cucumis sativus* —3, *Cucurbita maxima* —3, *Cucurbita sp.* (Naples Squash) —3, *Cucurbita sp.* (Seed from Gourd on Egyptian Vine) —3, *Cucurbita sp.* (var. *medullosa*)? —3, *Cuphea miniata* —3, *Cupressus sempervirens stricta* W, *Curculigo capitulata* —4, *Cyanopsis* (*tetragonoloba*)? —4P, *Cyathea sp.* (mixed) —4, *Cynoglossum amabile* —4P, *Cynoglossum linifolium* —4P, *Cynoglossum sp.* (blue) —3, *Cyperus alternifolius* —4, *Cyphomandra betacea* E, *Cypripedium acaule* (Lady slipper) —3, *Cytisus austriacus* —4, *Cytisus canariensis* —3, *Cytisus battandieri* —4, *Cytisus hirsutus* WE, *Cytisus multiflorus* +4, *Cytisus nigricans* —4, *Cytisus praecox* WE, *Casuarina sp.* —3, *Catananche caerulea* (Cupid's Dart) —3, *Cathartia villosa* —4, *Cautlea lutea* —4, *Cedrus libani* —4, *Celastrus orbiculatus* —3, *Celosia plumosa* —3, *Celsia arcturus* —4, *Celtis occidentalis* —3, *Centaurea cyanus* (Double cornflower) —3, *Centaurea montana* (Mountain Bluet) —3, *Centaurea moschata* —4, *Centaurea chilensis* —4, *Centranthus angustifolius* —4, *Centranthus sp.* —4, *Centrosema plumieri* —4P, *Centrosema pubescens* —4P, *Cephalaria tatarica* —4, *Cerastium tomentosum* —3, *Ceratonia siliqua* —3, *Ceratotheca triloba* —4, *Cercis canadensis* —3, *Cercis chinensis* +4, *Cercis occidentalis* —4P, *Cercis siliquastrum* +3R, *Cestrum sp.* —4P, *Chaenomeles japonica* +4, *Chaenomeles sp.* —4, *Chaenostoma (grandiflorum)*? —4, *Chamaecyparis lawsoniana* —4P, *Chamaelaucium ciliatum* —4, *Cheiranthus allionii* —3, *Chenopodium ambrosioides* —3, *Chlorophytum (amoniense)*? —4P, *Chrysanthemum carinatum* —4P, *Chrysanthemum cinerariaefolium* —3, *Chrysanthemum sp.* 3, *Chrysobalanus icaco* —4P, *Chrysophyllum cainito* —4P, *Chrysophyllum oliviforme* —4P, *Cicer arietinum* —4, *Cipadessa baccifera* —4P, *Cipadessa fruticosa* E, *Citrullus vulgaris* (Watermelon Chilean Black) —3, *Citrullus vulgaris* (Watermelon Dixie Queen) +3, *Citrullus vulgaris* (Watermelon Irish Gray) —3, *Citrullus vulgaris* (Watermelon Kafr-Hakim) —3, *Citrullus vulgaris* (Watermelon Klekly Sweet) —3, *Citrus reticulata* (Tangerine) —3, *Cladanthus arabicus* —4, *Cladrastis lutea* (Yellow-Wood Tree) —3R, *Clarkia elegans* —3, *Clarkia sp.* —3, *Claytonia virginica* —4, *Cleome spinosa* —3, *Clerodendron siphonanthus* +4P, *Clerodendrum squamatum* +4P, *Clerodendrum speciosissimum* —4P, *Clidemia hirta* W, *Chlorophytum sp.* —4P, *Clusia rosea* —4P, *Clusia sp.* —4P, *Coccolobis sp.* —4P, *Cochlospermum sp.* —4P, *Cochlospermum vitifoluim* —4P, *Cocos nucifera* —3, *Coffea arabica* (green beans) —4, *Cytisus purpureus* —4, *Cytisus ratisbonensis* WE, *Cytisus scoparius* W, *Cytisus scoparius andreanus* —4, *Cytisus (sinensis)*? —4P, *Cytisus sp.* —4, *Cytisus supinus* WE,. *Dahlia sp.* —3, *Dalbergia sissoo* —4P, *Daphne mezereum* +4, *Daphniphyllum humile* W, *Daphniphyllum macropodum* W, *Darlingtonia (californica)*? —4, *Datura discolor* 3R, *Datura factuosa* W, *Datura ferox* 3R, *Datura inoxia* +3, *Datura metel* —3, *Datura stramonium* +3, *Daucus sativa* —3, *Daucus sp.* var. *carota* —3, *Delonix regia* —4P, *Delphinium bellamosum* —4, *Desmanthus virgatum* —4P, *Desmodium cajanifolium* —4P, *Desmodium canum* —4P, *Desmodium cinereum* —4P, *Desmodium diffusum* —4P, *Desmodium dillenii* —4P, *Desmodium discolor* —4P, *Desmodium distortum* —4P, *Desmodium intortum* —4P, *Desmodium gyrans* —4P, *Desmodium gyroides* —4P, *Desmodium heterocarpum* —4P, *Desmodium laburnifolium* W, *Desmodium nicaraguense* —4P, *Desmodium polycarpum* —4P, *Desmodium purpureum* —4P, *Desmodium sp.* —4P, *Desmodium stipulaceum* —4P, *Desmodium supinum* —4P, *Desmodium tiliaefolium* —4P, *Desmodium umbellatum* —4P, *Desmodium uncinatum* —4P, *Desmodium uncinatum* (Kana) —4P, *Desmodium uncinatum* (Pensacola) —4P, *Desmodium uncinatum* (Ulupalakica) —4P, *Desmodium virgatum* —4P, *Dianthus chinensis* —3, *Dianthus heddewigii* —4P, *Diascia barberae* —4, *Dicentra eximia* —3, *Dicranostigma franchcrianum* —4, *Dicranostigma lactucoides* —4, *Dictamnus sp.* (mixed) —4, *Didiscus caerulea* —3, *Digitalis mertonensis* —4, *Dillenia indica* —4P, *Dimorphotheca sp.* —4, *Dimorphotheca sp.* (White African Daisy) —3, *Dipsacus fullonum* —4, *Disanthus cercidifolia* +4P, *Dissotis incana* —4, *Dolichos debilis* +4P, *Dolichos falcatus* —4P, *Dolichos (halei)*? —4P, *Dolichos lablab* E, *Dolichos lablab* (albiflorus) +4P, *Dolichos lablab* (Brown seed) +4P, *Dolichos lablab* (Hyacinth bean) +4, *Dolichos lablab* (var. *purpureum*)? W, *Dolichos soudanensis* +4, *Dolichos sp.* —4, *Dolichos sp.* (Princess Helene) +4, *Dolichos sp.* (var. *lablab*)? +4P, *Dombeya natalensis* —3, *Dracocephalum (montana)*? —3, *Drypetes glauca* —4P, *Duchesnea indica* —3, *Dyckia sp.* (Blend) —3. *Ec-*

cremocarpus scaber —3, *Echeveria sp.* (Mixed) —3, *Echinocereus engelmanni* —4, *Echinochloa frumentacea* —4, *Echium sp.* —4, *Ehretia microphylla* —4P, *Elaeis guineensis* —4P, *Elaphrium simaruba* —4P, *Enterolobium cyclocarpum* —4P, *Enterolobium saman* —4P, *Ephedra altissimo* —3, *Epidendrum o'brienianum* —3, *Epilobium nummularifolium* —3, *Eragrostis abyssinica* —4, *Eranthis hyemalis* —3, *Eremurus sp.* —3, *Erica cinerea* —3, *Eryngium sp.* (Mixed) —4, *Erythrine berteroana burare* +4P, *Erythrina corallodendrum* +4P, *Erythrina crista-galli* +4, *Erythrina glauca* +4P, *Erythrina poeppigiana* +4P, *Erythrina sumutrana* +4P, *Eschscholzia sp.* (Mixed) —3, *Eschscholzia sp.* (Mixed Cal. Poppy) —4P, *Eschscholzia sp.* (Orange) —3, *Eucalyptus* (Gum Tree) —3, *Eucalyptus dives* —4P, *Eugenia uniflora* W, *Eulalia (miscanthus)*? —3, *Euonymus alatus* —4P, *Euonymus americanus* —4P, *Euonymus atropurpureus* +4P, *Euonymus europaeus* (skinned) WE, *Euonymus europaeus* (Whole fruit) +4P, *Euonymus yedoensis* —3, *Euphorbia corollata* —3, *Euphorbia (didyma)*? W, *Euphorbia heterophylla* —4, *Euphorbia (longana)*? W, *Euphorbia myrsinites* —4P, *Euphorbia polychroma* —4, *Euphorbia sp.* W, *Euphorbia variegata* —3, *Euterpe globosa* —4P, *Fagopyrum esculentum* —3, *Festuca elatior* —3, *Ficus elastica* —4, *Ficus sp.* —3, *Filipendula hexapetala* (Single) —4, *Flacourtia indica* —4P, *Foeniculum vulgare* —3, *Freesia sp.* —4, *Fuchsia sp.* —4, *Fungus* (from Birch tree) —3. *Gaillardia grandiflora* —3, *Gaillardia sp.* (yellow) —4, *Galactia filiformis* +4P, *Galactia striata* +4P, *Galactia sp.* +4P, *Galax aphylla* —4, *Garcia nutans* —4P, *Gardenia jasminoides* —4, *Gardenia jasminoides Ellis* —3, *Gaultheria procumbens* —4, *Gaura lindheimeri* —4, *Genipa americana* +3, *Genista aethnensis* +4, *Genista canariensis* WE, *Genista fragans* —4, *Genista germanica* —4, *Genista monosperma* —4, *Genista pilosa* —4, *Genista radiata* —4, *Genista sagittalis* +4, *Genista tinctoria* —4, *Gentiana acaulis* —3, *Geum* (Lady Stratheden)? —3, *Gilia densiflora hybridus* —4, *Gillenia trifoliata* —4, *Ginkgo biloba* —3R, *Glaucium sp.* (peerless hybrids) —4, *Gleditschia japonica* —4P, *Gleditschia sinensis* —4P, *Gleditschia triacanthos* (Honey or Sweet Locust) —3R, *Gleditschia triacanthos (inermis)* —3, *Gliricidia sepium* —4P, *Globularia cordifolia* —4, *Glossostemon bruguieri* —3, *Glycine max.* +3, *Glycine soja* (Biloxi) W, *Glycine soja* (Otootan) —4P, *Glycine soja* (Soybeans-Hispidia) —3, *Godetia (Shamini)*? —3, *Gomphrena globosa* —3, *Gossypium barbadense* —4, *Gossypium hirsutum* —4P, *Gossypium sp.* (Cotton) —4, *Grevillea robusta* —4, *Guazuma ulmifolia* —4P, *Gymnocladus dioica* —4, *Gypsophylla elegans* —3, *Gypsophylla sp.* —4. *Haematoxylon campechianum* —3, *Hebenstretia comosa* —4, *Hedychium sp.* (Mixed) —4, *Helenium sp.* (Yellow) —4, *Helianthemum* (fine blend) —4, *Helianthus annuus* —3, *Helianthus sp.* —4, *Heliconia bihai* —4P, *Heliconia latispatha* —4P, *Heliotropium (lemoines)*? —3, *Heliotropium sp.* —4, *Helipterum roseum* —3, *Helleborus niger* —3, *Hepatica sp.* —4, *Heracleum mantegazzianum* —4, *Hesperis matronalis* —3, *Hesperis* (Rocket Garden) —3, *Heuchera sp.* —4, *Hevea brasiliensis* —3. *Hibiscus cannabinus* —4, *Hibiscus esculentus* (Okra-Dwarf green) —3, *Hibiscus esculentus* (Okra-Dwarf kauir) —3, *Hibiscus esculentus* (Okra-White Velvet) —3, *Hibiscus mutabilis* —4P, *Hibiscus sabdariffa* —3, *Hockea sp.* +3, *Homeria collina* —3, *Hordeum vulgare* (Barley-Aleysinian 13) —4, *Hordeum vulgare* (Barley-Beladi 16) —4, *Hordeum vulgare* (Barley-Beladi, Giza 73) —4, *Hordeum vulgare* (Barley-Nabawoi-2) —4, *Hovenia dulcis* —4, *Hunnemannia sp.* —4, *Hura crepitans* +4P, *Hylocereus (trigonus)*? —4P, *Hymenocallis declinata* —3, *Hyoscamus muticus* —3, *Hyoscyamus niger* —3, *Hypericum olympicum* —4. *Iberis sempervirens* —3, *Iberis umbellata* —3, *Ilex aquifolium* —4P, *Impatiens sultanii* (Hybrids) —3, *Incarvillea variabilis* —3, *Indigofera anil* —4P, *Indigofera arrecta* —4P, *Indigofera cordifolia* —4P, *Indigofera cytisoides* —4P, *Indigofera dosua* —4P, *Indigofera endecaphylla* (Hawaian Zoebistch var.) —4P, *Indigofera grandulosa* —4P, *Indigofera hirusta* (Early) —4P, *Indigofera hirusta* (Late) —4P, *Indigofera kirilowii* —3, *Indigofera linifolia* —4P, *Indigofera mucronata* —4P, *Indigofera pilosa* —4P, *Indigofera pulchella* —4P, *Indigofera sp.* —4P, *Indigofera subulata* —4P, *Indigofera suffruticosa* —4P, *Indigofera sumatrana* —4P, *Indigofera teysmanni* —4P, *Indigofera tinctoria* —3, *Ipomoea purpurea* —3, *Iris filifolia* —4, *Iris kaempferi* —3, *Iris sp.* (White Iris) —4, *Iris sp.* (Yellow Iris) —4, *Iris sp.* (Wedgwood) —4, *Isatis sp.*, —4, Italian Pole Bean (Pitt) +3. *Jambos jambos* WE, *Juniperus chinensis* —3, *Juniperus communis* —4P, *Kigelia aethiopica* —4P, *Kitalbela vitifolia* —4, *Kleinhavia hospita* —4P, *Kniphofia uvaria* —4, *Kochia childsii* —3, *Kolkwitzia amabilis* —4, *Krameria ixina* WE. *Laburnum alpinum* —4, *Laburnum anagyroides* —4, *Laburnum watereri* —3, *Lactuca capitata* —3, *Lactuca intybacea* —4P, *Lactuca romana* —4, *Lactuca sativa* —3, *Lactuca scariola* (var. *Oleifera*)? —4, *Lactuca sp.* var. *Longifolia* —3, *Lagerstromeia indica* —4, *Lantana sp.* —4, *Larix leptolepis* —4, *Lathyrus hirsutus* +4P, *Lathyrus latifolius* +4R, *Lathyrus maritimus* +4, *Lathyrus odoratus* (Cuthbertson-Evelyn) +4, *Lathyrus odoratus* (Cuthbertson-Frank G.) +4, *Lathyrus odoratus* (Cuthbertson-Giant Spencer) +4, *Lathyrus odoratus* (Cuthbertson-Janet) +4, *Lathyrus odoratus* (Cuthbertson-Little Sweetheart) +4, *Lathyrus odoratus* (Cuthertson-Lois) +4, *Lathyrus odoratus* (Cuthbertson-Marion) +4 *Lathyrus odoratus* (Cuthbertson-Mixed Strain) +4, *Lathyrus odoratus* (Cuthbertson-Tommy) +4, *Lathyrus odoratus* (Multiflora mixed) +4, *Lathyrus odoratus* (Sweet Pea) +4R, *Lathyrus odoratus* (White Sweet Pea) +3, *Lathyrus sp.* (White Pearl) +4, *Lavandula vera* —3, *Lavatera sp.* —4, *Layia elegans* —4, *Lens esculenta* +3, *Leonotis leonurus* —4, *Lespedeza bicolor* —4P, *Lespedeza thunbergii* —4P, *Leucaena glauca* —4P, *Libidibia (punctata)*? —4P, *Lilium philippinense* —4P, *Limonium bonduellii* —3, *Limonium sp.* —4, *Linaria*

maroccana –3, *Linaria sp.* –4, *Linum grandiflorum rubrum* –3, *Linum narbonense* –4, *Linum usitatissimum* (Flax-Dirae Monarch) –4, *Linum usitatissimum* (Flax-Giza 3) –4, *Linum usitatissimum* (Flax-Local Hindi) –4, *Lobelia erinus* –3, *Lobelia sp.* (Dark Blue) –4, *Lopezia coronata* –4, *Lotus corniculatus* –4P, *Lotus (mascaensis)*? –4, *Lotus sp.* –4 *Luculia gratissima* –3, *Luffa cylindrica* –3, *Lupinus angustifolius* –4P, *Lupinus hartwegii* –4P, *Lupinus luteus* +4P, *Lupinus polyphyllus* –3, *Lupinus polyphyllus* (carmineus)? –4P, *Lupinus sp.* –3 *Lychnis coronata* –3, *Lycopersicon esculentum* –3 *Lysimachia vulgaris* –4, *Lythrum salicaria* –3. *Maackia amurensis* +4, *Macleaya cordata* –4, *Macroptylium lathyroides* –4P, *Malacantha warneckiana* –4P, *Malpighia punicifolia* –4P, *Mammea americana* –4P, *Malus domestica* –3, *Malus domestica* (Apple seed yellow) –4P, *Mandevilla suaveolens* –3, *Mangifera indica* +3, *Mangosteen sp.* –4P, *Manihot glagiovii* +3, *Matricaria chamomilla* –3, *Matricaria parthenoides* –4R, *Matricaria capensis* –4R, *Matthiola bicornis* (Night scented stock) –4R, *Matthiola sp.* –3, *Maurandya erubescens* –3, *Meconopsis baileyi* –3, *Meconopsis baileyi* (Sky Blue) –4P, *Medicago arabica* –4P, *Medicago intertexta* –4P, *Medicago hispidia* –4P, *Medicago sativa* –4P, *Medicago sativa* (Alfalfa) –3, *Medicago sativa* (African Alfalfa –4P, *Medicago sativa* (Arizona, Chilean Alfalfa seed) –4P, *Medicago sativa* (Buffalo Alfalfa) –4P, *Medicago sativa* (Caliverde Alfalfa) –4P, *Medicago sativa* (Alfalfa Egypt) –4P, *Medicago sativa* (Indian Alfalfa seed) –4P, *Medicago sativa* (Labontan Alfalfa) –4P, *Medicago sativa* (North Carolina Alfalfa) –4P, *Medicago sativa* (Ranger Alfalfa seed) –4P, *Medicago sativa* (Uruguay Clove Alfalfa) –4P, *Medicago sativa* (var. *gaetula*) –4P, *Medicago sp.* –4P, *Melastoma sp.* –3, *Melicocca bijuga* WE, *Melilotus alba* –4P, *Mentzelia lindleyi* –4R, *Mesembryanthemum criniflorum* –3, *Mimosa (bimucronata)*? –4P, *Mimosa pudica* –3, *Mimosa sepiaria* +4, *Mimulus sp.* (Monkey Flower) –4P, *Mimulus tigrinus* –3, *Mina lobata* –3, *Mirabilis jalappa* –3, *Monarda fistulosa* –3, *Monsonia speciosa* –3, *Moraea irioides* –3, *Morinda citrifolia* –4P, *Moringa oleifera* –3, *Moringa sp.* –4P, *Mucuna prurita* WE, *Musa (gloriosa)*? –4P, *Musa sp.* –4P, *Musa velutina* –3, *Myosotis alpestris* –3, *Myosotis macrophylla* –3, *Myristica fragans* –3, *Myroxylon pereirae* –4P. *Nageia coriacea* –4P, *Nemesia sp.* (Large flowering hybrids) –3, *Nemophilia insignis.* (Cal. Blue Bell, Baby Blue-eyes) –3, *Nemophilia menziesii* –4, *Nepeta mussinii* –3, *Nertera granadensis* (var. *N. depressa*)? –4, *Nicotiana acuminata* –3, *Nicotiana affinis* (Daylight White) WE, *Nicotiana affinis* (White) –4P, *Nicotiana rustica* –3, *Nicotiana tabacum* –3, *Nierembergia caerula* –3, *Nigella damascena* –4R, *Nigella sativa* –3. *Ochroma lagopus* –4P, *Ochroma pyramidale* –4P, *Ochrosia elliptica* –4P, *Ocimum basilicum* –3, *Ocotea leucoxylon* +4P, *Oenothera lamarckiana* –3, *Omphalodes amabile* (Blue) –4R, *Omphalodes linifolia* (White) –4R, *Oncidium sp.* –4P, *Oncoba echinata* –4P, *Onobrychis viciaefolia* –4P, *Onosma tauricum* –4, *Opuntia macrorhiza* –4, *Ormosia dasycarpa* –4P, *Ormosia jamaicensis* –4P, *Ormosia panamensis* –4P, *Ormosia parvifolia* –4, *Oryza sativa* (Milled Rice–Nabatal Asmar) –4, *Oryza sativa* (Hulled Rice–Nabatal Asmar) –4, *Oryza sativa* (Yabani Rice–Hulled) –4, *Oryza sativa* (Yabani–Milled) –4, *Oryza sativa* (Yabani–Paddy Rice) –4, *Oryza sativa* (Yabani Pearl–Paddy Rice) –4, *Oryza sativa* (Yabani Pearl–Hulled) –4, *Oryza sativa* (Yabani Pearl–Milled Rice, Par-boiled) –4, *Oxalis valdiviensis* –4. *Pachyrhizus erosus* –4P, *Pachyrhizus sp.* –4P, *Pakistan seeds:* seeds in husk –3, and seeds from village –3, *Panicum miliaceum* –4, *Papaver nudicaule* (Poppy nudicaule) –4P, *Papaver nudicaule* (Poppy nudicaule–double mixed) –4P, *Papaver nudicaule* (Poppy nudicaule–single mixed) –4P, *Papaver nudicaule* (Poppy nudicaule, Gartford Gts., mixed) –4P, *Papaver nudicaule* (Iceland Poppy) –4, *Papaver orientale* (Poppy–orientalis, mixed) –4P, *Papaver orientale* (Poppy–orientalis, Aksarbes) –4P, *Papaver orientale* (Poppy–orientalis, Princess Victoria Louisa, salmon) –4P, *Papaver orientale* (Poppy–orientalis, Beauty of Livermore) –4P, *Papaver orientale* (Poppy–orientalis Cerise Beauty) –4P, *Papaver orientale* (Poppy–orientalis, Cheerio) –4P, *Papaver orientale* (Poppy–orientalis, Rose Beauty) –4P, *Papaver orientale* (Poppy–orientalis, Cedar-Hill) –4P, *Papaver orientale* (Poppy–orientalis, Glowing Amber) –4P, *Papaver orientale* (Oriental Poppy) –4, *Papaver rhoeas* –3, *Papaver sp.* (Poppy–Shirley double-mixed) W, *Papaver sp.* (Poppy–Shirley single-mixed) –4P, *Papaver sp.* (Poppy) –4, *Parkia biglandulosa* +4P, *Parkia speciosa* +4P, *Parkinsonia aculeata* –4R, *Parmentiera edulis* –4P, *Parthenocissus tricuspidata* –4, *Passiflora edulis* –4P, *Passiflora sp.* (Blend) –4, *Pastinaca sativa* –3, *Pelargonium zonale* (Geranium) +4R, *Peltophorum brasiliense* –4P, *Peltophorum ferrugineum* –4P, *Peltophorum sp.* –4P, *Pennisetum glaucum* –4, *Penstemon* (Giant Floradale) –3, *Penstemon barbatus* –3, *Penstemon barbatus* (Chelone barbatus mixed) –4R, *Penstemon glaber* –4R, *Peperomia sp.* –4, *Perilla frutescens* var. *crispa* –4, *Periploca sp.* –4, *Persea americana* –3, *Persea persea* (L. Cockerell) WE, *Petroselinum crispum* –3, *Petroselinum hortense* –3, *Petunia sp.* (Hybrids) –3, *Phacelia campanularia* –3, *Phaeomeria speciosa* WE, *Phalaris canariensis* –4, *Phaseolus aconitifolius* WE, *Phaseolus angularis* –4P, *Phaseolus aureus* –4P, *Phaseolus aureus* (Mung Bean) –3, *Phaseolus calcaratus* E, *Phaseolus calcaratus* (red seed) –4P, *Phaseolus caracalla* –4P, *Phaseolus coccineus* (Runner Bean–Sutton's Best of All) +4, *Phaseolus coccineus* (*Phaseolus coccineus* (Runner Bean–Sutton's Scarlet) +4, *Phaseolus coccineus* (Scarlet Runner Pole Bean) +4R, *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*–Frozen Cold Seal) –3, *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*–Dwarf Bean Black Valentine) –3, *Phaseolus lunatus* var. *macrocarpus* (*P. limensis*–Triumph) +3, *Phaseolus mungo* –4P, *Phaseolus ricciardianus* –4P, *Phaseoolus sp.* (Best Pole

Lima Bean) −3, *Phaseolus sp.* (Contender) +3, *Phaseolus sp.* (Great Northern Bean) +3, *Phaseolus sp.* (Mazakan) +3, *Phaseolus sp.* (McLeod) +3, *Phaseolus sp.* (Michelite Pea Bean) +3, *Phaseolus sp.* (Mont-Calm) +3, *Phaseolus sp.* (Pinto Bean) +3, *Phaseolus sp.* (Plentiful) +3, *Phaseolus sp.* (Pole Bean, Blue Lake, Black Seeded) −3, *Phaseolus sp.* (Pole Bean, Blue Lake, White Seeded) +3, *Phaseolus sp.* (Pole Bean—Italian) +3, *Phaseolus sp.* (Pole Bean Lazy Wife's) +3, *Phaseolus sp.* (Pole Bean McCaslan) +3, *Phaseolus sp.* (Puregold) +3, *Phaseolus sp.* (Shell Bean) +3, *Phaseolus sp.* (Speckled Bean) +3, *Phaseolus sp.* (Speckled Bean—Florida) E, *Phaseolus sp.* (White Bean Calif. Small) +4R, *Phaseolus sp.* (Yellow Eye Bean—Old York) +3, *Phaseolus vulgaris* (Cherokee Wax) +3, *Phaseolus vulgaris* (Climbing French Bean) +4, *Phaseolus vulgaris* (Dwarf Horticultural Bean) +3, *Phaseolus vulgaris* (Kentucky Wonder Pole Bean) +4R, *Phaseolus vulgaris* (Kidney Bean) +3, *Phaseolus vulgaris* (Kidney Wax Round Pod) +3, *Phaseolus vulgaris* (Pole Bean) +3, *Phaseolus vulgaris* (Surecrop Stringless Wax) +3, *Phaseolus vulgaris* (The Masterpiece) +4, *Phaseolus vulgaris* (The Prince) +4R, *Phaseolus vulgaris* var. *humilis* (Brittle Wax Pod Bean) +4R, *Phaseolus vulgaris* var. *humilis* (Wax Pod Bush Bean) +4R, *Phellodendron amurense* −3, *Phellodendron lavallei* −3, *Phleum pratense* −3, *Phlox nana (compacta)*? −3, *Phoenix dactylifera* −4, *Phoenix sp.* −3, *Phoenix sp.* (Black Date) −4, *Physalis alkekengi* −4, *Physalis franchetii* (Chinese Lantern) E, *Physalis sp.* (Ground Cherry) −4, *Physostegia virginiana* −3, *Physostigma venenosum* −3, *Pimpinella anisum* −3, *Pinus cembroides monophylla* −3, *Pinus griffithii* −4R, *Piper nigrum* −4P, *Piper sp.* −3, *Pistacia sp.* −3, *Pisum arvense* +4R, *Pisum sativum* (2810) +3, *Pisum sativum* (2817) −3, *Pisum sativum* (Lincoln) +3, *Pisum sativum* (Little Marvel) +3, *Pisum sativum* (Montana Monarch) +4R, *Pisum sativum* var. *humile* (Dwarf Sugar Pea) +4R, *Pisum sativum* var. *humile* (Early Alaska) +4R, *Pisum sativum* var. *humile* (Latton's Progress) +4R, *Pisum sp.* −4, *Pisum sp.* (Canada Field Peas) +4, *Pisum sp.* (Cow Peas) −4, *Pithecellobium grandiflorum* WE, *Plumeria rubra* −4P, *Podocarpus coriacea* −4P, *Poinciana pulcherrima* −4P, *Polialthia tuberosa* −4P, *Polianthes sp.* WE, *Pongamia* (pinnata)? −3, *Portulaca sp.* −3, *Potentilla warrensii* −3, *Primula auricula* −4R, *Primula obconica* −4R, *Primula (stellata)*? −4R, *Primula veris* WE, *Pritchardia pacifica* −4P, *Prunus americana* WE, *Prunus avium* −3, *Prunus cerasifera* −3, *Prunus mahaleb* −3, *Prunus serotina* −4P, *Prunus sp.* −3, *Psidium cattleianum* −3, *Psidium guajava* −4P, *Psidium littorale* −4P, *Psidium microphyllum* −4P, *Psophocarpus tetragonolobus* +4P, *Psychotria bacteriophylla* −4, *Psychotria sp.* −4P, *Ptelea trifoliata* −4, *Pterocarpus indicus* −4P, *Pterocarpus macrocarpus* +4P, *Pterocarpus sp.* +4P, *Pueraria phaseoloides* −4P, *Pueraria thunbergiana* −4P, *Punica granatum* −3, *Pyrus sp.* −3, *Quamoclit lobata* −3, *Quassia amara* +4P, *Randia formosa* +4P, *Ranunculus asiaticus* −3, *Ranunculus sp.* −4, *Raphanea guianensis* WE, *Raphanus sativus* (Radish red, White tipped) −3, *Raphanus sativus* (Scarlet Globe) −3, *Reseda odorata* −3, *Reseda sp.* −3, *Rhamnus californica* −4P, *Rhamnus cathartica* −4P, *Rhododendron hippophaeoides* −4P, *Rhodendron sp.* −4, *Rhynchosia minima* WE, *Ricinis communis* (Hindi 12) +3, *Ricinis communis* (Hindi 21) −3, *Robinia fertilis* +4, *Robinia kelseyi* +3, *Robinia neo-mexicana* +3, *Robinia pseudoacacia* +3R, *Rubus glaucus* +4P, *Rubus rosafolius* −4P, *Rudbeckia serotina* −3. *Sabal sp.* −4P, *Sabinea sp.* −4P, *Saccharum officinarum* −4R, *Saintpaulia ionantha* −4R, *Salpiglossis sinuata* −3, *Salvia argentea* −3, *Salvia haematodes* −3R, *Salvia sp.* −3, *Sapindus sp.* −4P, *Saxifraga cespitosa* −4P, *Scabiosa atropurpurea* −3R, *Scabiosa fischeri* −3, *Scheelea zonensis* −3, *Schizanthus sp.* (Butterfly flower) −3, *Scutellaria ventenatii* −4P, *Securidaca (diversifolia)*? +4P, *Senechio elegans* −4, *Sequoia sempervirens* +4P, *Sesamum indicum* −3, *Sesamum indicum* (Sesame—Griza red) −4, *Sesamum indicum* (Sesame—Griza white) −4, *Sesamum indicum* (Sesame—Nabatat 8) −4, *Sesamum indicum* (Sesame—Nabatat 21) −4, *Setaria italica* var. *rubrofructa* −4R, *Sidalcea sp.* (Hybrids mixed) −3, *Sinningia speciosa* −4R, *Siphonanthus indicus* +4P, *Solandra grandiflora* −4P, *Solanum auriculatum* −4P, *Solanum aviculare* −4P, *Solanum (guitaense)*? +4P, *Solanum marginatum* −4P, *Solanum melongena* var. *esculentum* (Eggplant—Black) −3, *Solanum melongena* var. *esculentum* (Eggplant—Globe) −3, *Solanum melongena* var. *esculentum* (Eggplant—White) −3, *Sophora affinis* −4P, *Sophora alopecuroides* +4P, *Sophora secundiflora* −3, *Sophora sp.* (Coral Bean) −4P, *Sophora viciifolia* −3, *Sorghum sudanense* −3, *Sorghum technicum* −4, *Sorghum vulgare* (Grain Sorghum—Ajaz 31) −4, *Sorghum vulgare* (Grain Sorghum—Fargo 35) −4, *Sorghum vulgare* (Grain Sorghum—Giza 25—local selection) −4, *Sorghum vulgare* var. *saccharatum* −4, *Sorghum vulgare* var. *sudanense* −4, *Spartium junceum* −4P, *Spathodea nilotica* +4P, *Sphenostylia stenocarpa* +4, *Spinacia oleracea* −3, *Stachys officinalis* −4, *Stachytarpheta jamaicensis* −4P, *Stahlia monosperma* (flesh around seed) WE, *Stahlia monosperma* (Seed) WE, *Statice bonduellii* −3, *Sterculia apetala* −3, *Sterculia foetida* −4P, *Stizolobium aterrinum* +4P, *Stizolobium deeringianum* −4P, *Stizolobium pruritum* −4P, *Stizolobium sp.* −4P, *Strychnos spinosa* −4P, *Stylosanthes bojeri* −4P, *Stylosanthes gracilis* −4P, *Sutherlandia frutescens* −4P, *Sutherlandia microphylla* −4P, *Swietenia mahogani* −3, *Swurtzia grandiflora* −4P, *Synsepalum dulcificum* −4P, *Syringa sp.* −4, *Tabebuia haemantha* −4P, *Tacca chantrieri* −4P, *Tacca sp.* −4P, *Tagetes erecta* −3, *Tagetes sp.* (Marigold orange) −4, *Tamarindo* +4P, *Tamarindus indica* +4P, *Taraxacum officinale* −3, *Taxus baccata* +4P, *Taxus cuspidata* −3, *Tecomaria stans* −4, *Tephrosia candida* −4P, *Tephrosia noctiflora* −4P, *Tephrosia sinapou* −4P, *Tephrosia toxicaria* −4P, *Tephrosia villosa* −4P, *Tephrosia vogeli* −4P,

Teramnus labialis —4P, *Teramnus repens* WE, *Teramnus sp.* —4P, *Teramnus uncinatus* —4P, *Teramnus volubilis* —4P, *Terminalia catappa* +4P, *Terminalia muelleri* +4P, *Thalictrum dipterocarpum* —3, *Theobroma cacao* —4P, *Thermopsis caroliniana* —3, *Thermopsis rhombifolia* —4P, *Thermopsis xylorrhiza* +4P, *Thurberia sp.* —3, *Thymus vulgaris* —3, *Tolly pot Palm* +4P, *Tonka* —3, *Trachymene caerulea* —4, *Trifolium alexandrinum* +4P, *Trifolium campestre* —4P, *Trifolium incarnatum* —4P, *Trifolium johnstonii* +4P, *Trifolium mexicanum* —4P, *Trifolium nigrescens* +4P, *Trifolium pratense* —4, *Trifolium repens* (Hillboro White) —4P, *Trifolium repens* (Landino Clover) —4P, *Trifolium repens* (White Dutch Clover) —3, *Trifolium sp.* —4P, *Trifolium subrotundum* +4P, *Trifolium subterraneum* +4P, *Trifolium xerocephalum* —4P, *Trigonella foenum-graecum* —3, *Triplaris americana* E, *Triticum sp.* (Wheat Beladi 116) —4, *Triticum sp.* (Wheat Beladi Abia) —4, *Triticum sp.* (Wheat Bouhi) —4, *Triticum sp.* (Wheat Dakar 49c) —4, *Triticum sp.* (Wheat Dakar 52) —4, *Triticum sp.* (Wheat Giza 139) —4, *Triticum sp.* (Wheat Hindi 62) —4, *Triticum sp.* (Wheat Hindi D) —4, *Triticum sp.* (Wheat Mabrouk) —4, *Tritoma uvaria* —3, *Trollius europaeus* —4P, *Tropaeolum majus* E, *Tropaeolum sp.* (Nasturtium) —4P, *Tropaeolum peregrinum* —4R. *Ulmus americana* —4R, *Urania lancinfolia* —4P, *Ursinia anethoides* —4. *Vachellia farnesiana* —4P, *Vachellia farnesiana* (Resin of) —4P, *Valeriana officinalis* —3, *Valerianodes jamaicensis* —4P, *Verbascum phoeniceum* —4, *Verbena sp.* —4, *Verbena venosa* —4R, *Veronica longifolia* —3, *Viburnum sargentii* —4P, *Vicia angustifolia* (Copenhagen) +4, *Vicia angustifolia* (Lyon) +4, *Vicia amphicarpa* +4, *Vicia articulata* +4, *Vicia atropurpurea* +4, *Vicia benghalensis* +4, *Vicia bythynica* +4, *Vicia calcarata* +4, *Vicia cracca* +4, *Vicia craspernia* +4, *Vicia dasycarpa* +4, *Vicia disperma* +4, *Vicia ervilia* +4, *Vicia faba* +4, *Vicia faba* (Beans, broad) +3, *Vicia faba* (Beans, broad espanialy) WE, *Vicia faba* (Dwarf beans, Broad Windsor) +4R, *Vicia faba* (Horse Beans) +3, *Vicia faba* (Horse or Windsor Beans) +3, *Vicia gemella* +4P, *Vicia graminea* E, *Vicia grandiflora* E, *Vicia hirsuta* +4, *Vicia hirsuta* (India) +4P, *Vicia hyrcanica* +4, *Vicia* (Ilūsgūinera)? —4, *Vicia lutea* —4, *Vicia menziesii* W, *Vicia monanthos* +4, *Vicia narbonensis* —4, *Vicia orobus* +4, *Vicia pannonica* +4, *Vicia sativa* +4, *Vicia sativa* (India) +4P, *Vicia tetrasperma* (India) +4P, *Vicia unijuga* +4, *Vicia villosa* +4, *Vicia villosa* (Hairy Vetch) +4, *Vicia sp.* (Fort Warren) —4, *Vigna luteola* —4P, *Vigna marina* —4P, *Vigna repens* WE, *Vigna sesquipedalis* —4P, *Vigna sinensis* —4P, *Vigna sinensis* (Braham) —4P, *Vigna sinensis* (Brown speckled) —4P, *Vigna sinensis* (Cowpea Philippines) —4P, *Vigna sinensis* (Mixed seeds) —4P, *Vigna sinensis* (White seeds) —4P, *Vigna sp.* —4P, *Vigna vexillata* —4P, *Vinca rosea* —4P, *Vinca sp.* —4, *Viola cornuta* —4R, *Viola tricolor* —3, *Vitex divaricata* —4P, *Vitis rotundifolia* —4R, *Vitis vulpina* —4R. *Wisteria floribunda* E, *Wisteria floribunda alba* +4P, *Wisteria frutescens* +4P, *Wisteria macrobotrys* +4P, *Wisteria macrostachya* —4, *Wisteria sinensis* (Blue) +3, *Wisteria sinensis* (White) +4P. *Zea mays* (Maize—Am. 4135) —4, *Zea mays* (Maize—Beladi) —4, *Zea mays* (Maize—Double Cross FI crop) —4, *Zea mays* (Maize—Double Cross 27 Hybrid seed) —4, *Zea mays* (Maize—Early Sabeini, White Flint) —4, *Zea mays* (Maize—Giza, Beladi) —4, *Zea mays* (Maize—Nab-El-Gama) —4, *Zea mays* (Maize—Single Cross 14) —4, *Zea mays* (Maize—Yellow Flint) —4, *Zea mays* var. *everta* —4, *Zea mays* var. *indurata* —4, *Zea mays* var. *rugosa* —3, *Zea mays* var. *saccharata* —4, *Zinnia sp.* —3, *Zizyphus mauritiana* —4P.

TABLE 3. *Seed extracts found to be hemolytic.*

Acacia greggii (Catclaw A), *Achras sapota, Aesculus hippocastanum, Agati grandiflora, Agave sp., Akebia quinata, Albizzia julibrissin, Albizzia lebbeck, Albizzia stipulata, Alysicarpus glumaceus, Alysicarpus rugosus, Amellus lychnitis, Anoda cristata, Anoda opoleup, Ardisia humilis, Ardisia solanacea, Arenaria montana. Bauhinia retusa, Bauhinia sp.* (White Flower). *Caesalpinia mimosoides, Cajanus cajan, Calcaea scauderes, Calonyction aculeatum, Calonyction grandiflora* (moonflower), *Calopogonium galactoides, Canavalia ensiformis, Celosia cristata, Centrosema plumieri, Cestrum nocturnum, Chrysophyllum oliviforme, Chrysophyllum sp., Clitoria sebestana, Clitoria sp., Clitoria ternatea, Coix lacrima-jobi, Collybia sp., Crotalaria tetragona. Desmodium nicaraguense, Dianthus barbatus, Dianthus caryophyllus, Dianthus chinensis, Dichelostemma pulchellum, Dierama sp., Digitalis* (gloxiniaeflora)?, *Digitalis purpurea, Dodecatheon meadia, Dolichos sp., Douglasia montana. Enterlobium cyclocarpum, Euonymus europaeus* (fruit part). *Gardenia grandiflora, Gilia rubra, Gilia sp., Guar. Hesperaloe parviflora. Kochia childsi, Koelreuteria paniculata. Leucocoryne ixioides, Lychnis oculata* (*Viscaria oculata*), *Lychnis sp., Maianthemum canadense, Medicago lupulina, Medicago teiboides, Medicago tuberculata, Mimusops elengi. Nicotiana affinis. Passiflora quadrangularis, Phaeomeria speciosa, Phaseolus adenanthus, Piptadenia peregrina, Pithecellobium brevifolium, Pithecellobium clypearia, Pithecellobium dulce, Polianthes tuberosa, Psophocarpus tetragonolobus. Rhus copallina, Rhus typhina. Samanea saman, Saponaria vaccaria, Sesbania aegyptiaca, Sesbania bispinosa, Sesbania cinerascens, Sesbania emerus, Sesbania grandiflora, Sesbania sesban, Sesbania sp., Sesbania speciosa, Solanum sodomeum, Swietenia candollei. Tabernaemontana citrifolia, Tecoma guadichaudi. Yucca whipplei.*

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Actively Acquired Tolerance in Fetal-Maternal Combinations: A Review**

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Two hypotheses have been developed as partial explanations of the protective mechanisms involved in Rh hemolytic disease. The first is based on the observations of Levine and others that Rh erythroblastosis is less likely to arise when the ABO blood group of the mother is serologically incompatible with that of the fetus. According to this theory, fetal blood cells are rapidly destroyed by maternal iso-antibodies and removed from her circulation before the sensitization has sufficient time to develop. The second, due to Owen, is based on the phenomenon of actively acquired tolerance. This theory asserts that maternal blood cells may enter the fetal blood stream during the critical period in which the organism "learns" to recognize its tissues as its own. Thus, an Rh negative fetus transfused *in utero* with Rh positive cells might be expected to be tolerant of further exposures to the Rh antigen in adult life. Owen's data, based upon sensitization of the Rh negative mother show a distinct correlation with the Rh type of her mother. The available evidence is not sufficient to exclude one of the two hypotheses, and it is possible that both operate. The data for fetal-maternal transfer of blood cells is far more conclusive than that for passage in the other direction. Definitive proof of tolerance to Rh antigens, however, requires furher study.

IMMUNOLOGICAL tolerance has been defined by Medawar[27] as a state of indifference or nonreactivity towards a substance that would normally be expected to excite an immunological response. Within the framework of the present review, the term "tolerance" will be employed in its broadest definition, including unresponsive states produced after the injection of anuclear organic material into a susceptible and immunologically competent host. In particular, this paper will examine the functional relationships which exist between the human mother and her fetus, the barriers that separate their circulations yet permit the free exchange of necessary nutritive and excretive products and the protective mechanisms that have evolved to permit viviparity to exist at all.

Numerous investigations have established that homotransplants are uniformly rejected except when exchanged between identical twins, agammaglobulinemics, persons or animals exposed to massive doses of X-irradiation and, in lower animals, individuals derived from isogenic (highly inbred) strains. Peer[37] has shown that human maternal-fetal and fetal-maternal skin transplants are more often rejected than accepted. How then does the fetus survive the long gestation period in a situation superficially analogous to homotransplantation?

Three factors have been proposed by Medawar[26] to explain the privileged position of the fetus. These are: (a) the anatomical separation of the fetus from the mother; (b) the antigenic immaturity of the fetus; and (c) the immunological inertness of the mother. Of these three, the first is probably the most decisive. Woodruff[47] in a series of experiments in which female rats and rabbits were pre-immunized with a homograft from the males with which they were to be mated, showed that grafts from the 15-16 day fetus were violently rejected as is characteristic of an adult second-set response. Unimmunized mothers, however,

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rejected the grafts slowly as expected of a first-set reaction. These findings, it was suggested, point to the conclusion that the anatomical separation of mother and fetus is sufficient and necessary to safeguard the mammalian fetus from the immunological hazards of pregnancy.

The exception to the rule is, of course, provided by the immunological syndrome known as hemolytic disease of the newborn or erythroblastosis fetalis caused as a result of maternal immunization to fetal red blood cell antigens. Thus, blood cells or some fraction of them must pass across the placenta in the direction of fetus to mother. In order to appraise the theory of Owen *et al.*[34] which invokes actively acquired tolerance as an explanation of the failure of some Rh negative women to produce anti-Rh antibodies against their Rh positive offspring, the evidence for the transplacental passage of red blood cells from mother to fetus will be examined.

Evolutionary Importance of the Placenta

The placenta has far-reaching significance when considered in the light of its evolutionary development. Among the Mammalia, only the Eutheria may be considered to be viviparous species. Other mammalians such as the therian marsupials deliver their young alive but at a tiny and immature stage. Beneath them on the mammalian tree are the prototherian duckbills and anteaters which, like their reptilian forebears, are oviparous. With some exceptions, non-placental animals are either oviparous or ovoviviparous. In the latter class, development is internal but takes place without the formation of a placenta or other means of obtaining nourishment from the mother. It should, therefore, be obvious that the placenta is instrumental not only in the maintenance of the embryo but also in its protection from immunological destruction.

This protection is perfect or nearly so in some species such as the horse in which hemolytic disease of the newborn is precipitated only after ingestion of antibody-laden colostrum or milk by the newborn colt (diathelic immunization). This situation holds also for swine, dogs and cattle although in the latter species newborn calves fail to show definite evidence of hemolytic disease even after the ingestion of colostrum containing maternal antibodies produced against the sire's blood and reactive *in vitro* with the calves' red blood cells. It appears that the mechanism which protects the newborn calf from hemolytic disease is the absorption or neutralization of ingested antibodies by tissue or soluble antigens, thus preventing these antibodies from reaching the calf's erythrocytes and causing their destruction.[17]

Physiology of the Human Placenta

The physiology of the placenta indicates that this organ should not be considered to function solely as a complex semipermeable membrane. Not only soluble foodstuffs but also viruses, antibodies and tumor cells have been shown to pass across the placenta into the fetal bloodstream. The placenta also possesses a hormonal function, itself secreting chorionic gonadotropin. Not so well known are the enzymatic activities of the placenta responsible for the detoxification of certain maternal substances such as histamine, the active transport of amino acids, some vitamins, iron, magnesium, cobalt and calcium against a concentration gradient and the production of fructose.[35] Dempsey[9] has reported the passage of particulate matter by the active transfer process of planocytosis which involves phagocytosis by the cells of the chorionic villi. A recent paper by Salvaggio *et al.*,[40] indeed, claims that trophoblastic tissue, itself, may pass into the fetal as well as the maternal circulation. If confirmed, these findings would be of utmost significance in explaining Peer's observation that grafts from mothers to offspring are sometimes retained indefinitely and almost always

have longer lifespans than those grafted from fathers to offspring.[37]

On the basis of pressure relationships between the fetal and maternal circulations, the direct passage of cells should always occur from fetus to mother. The infant's arterial pressure at birth is 60-70 mm. Hg while the venous pressure in the umbilical cord is 30-40 mm. Hg. This would make the capillary pressure in the villi about 45 mm. Hg. The pressure of maternal blood in the intervillous space with the uterus at rest is about 10 mm. Hg. While this pressure rises markedly with uterine contractions, a proportionate rise in fetal pressure must also occur.[33]

Evidence for Materno-Fetal Transfer of Red Blood Cells

Several studies have been undertaken in the past fifteen years attempting to ascertain whether red blood cells from the mother can enter the circulation of the fetus. Several of these were performed before Owen published his "tolerance to Rh" theory in 1954[34] and although such information is necessary in order for tolerance to be considered as a mechanism, the investigations were undertaken for quite different reasons.

The first of these experiments was reported in 1946 by Hedenstedt and Naeslund[14] who injected elliptocytic blood into two pregnant women. Elliptocytes were noted in the blood of one of the infants at birth. In this case the placenta had many sites of bleeding and infarction. Encouraged by these results, Naeslund and his collaborators[31, 32] continued their work with red blood cells tagged with radioactive phosphorous (P^{32}) or iron (Fe^{59}). One of the six infants of the first series and four of seven in the second showed significant amounts of radioactivity in the blood suggesting that radioactive blood cells had crossed the placenta.

On the other hand, Martland and Martland[24] fortuitously were able to examine the blood of two pregnant women killed by carbon monoxide. Although they exhibited carboxyhemoglobin saturations of 48 per cent and 88 per cent respectively, the fetal blood (5 months and 9 months) contained none.

Mengert *et al.*[28] in 1955 attempted to repeat the experiments of Naeslund with red cells tagged with Fe^{59}. Because of the technical problems encountered in preparing the tagged cells for radiation counts, Mengert was quite skeptical about the value of the results. In view of the pressure relationships between the two circulations, it seems unlikely that upwards of 30 ml. of maternal blood in one case could have entered the fetal bloodstream. This amount would be equal to about 1/10 of the total neonatal blood volume and would have been exchanged over a period of 16.5 hours. With the development of nearly routine procedures employing Cr^{51}-labeled red cells, the measurement of total blood volume has been greatly simplified and improved. The superiority of Cr^{51} over other radioactive labels in the study of erythrocyte life span lies in the fact that it is not re-utilized following red cell destruction and it is eliminated from the circulation when lost by elution from viable cells.[16] Repetition of these experiments with Cr^{51} would, therefore, appear to be more feasible than previous methods.

Because Mengert, himself, doubted the validity of his results, he was led to inject sickle-trait cells into pre-parturient mothers. In one case, 400 ml. of sickle-trait blood was transfused 21 hours before delivery and 0.0245 ml. of whole maternal blood was estimated to have transversed the placenta into the child by the time of birth. Similar qualitative results were reported in a second experiment.

In a similar study, Macris, Hellman and Watson[23] infused sickle-trait cells into 25 pregnant women shortly before delivery and noted transfer in three cases. Inter-

estingly, in one of nature's experiments, sickle-trait cells were not found in the blood of an infant delivered from a sickle-trait mother.

These represent all of the materno-fetal transfer experiments with red blood cells reported to date. As can be seen, the results are difficult to interpret because the number of variables is so large. All of these results are based on experimental transfusions given when the mother was in labor and hence at the time when placental injury is most likely to occur. Also the cells were infused in a citrate medium which could conceivably affect placental permeability. It should also be recalled that the two types of natural experiments with carbon monoxide-exposed and sickle-trait cells were negative.

No experiments have yet been reported utilizing the technics of fluorescent antibody, mixed cell agglutination or differential agglutination (Ashby method) in the study of this aspect of the problem.

Evidence for Feto-Maternal Transfer of Red Blood Cells

The first suggestion that fetal hemorrhage into the maternal circulation unrelated to placental or umbilical cord accidents could be the cause of neonatal anemia was made by Wiener[46] in 1948. Proof of this hypothesis was furnished by Chown[4] in 1954 from a study of an infant suffering from moderately severe anemia at birth. By means of differential agglutination, he was able to prove conclusively the presence of fetal erythrocytes in the mother. Evidently loss of fetal cells had occurred over a fairly long period since Chown estimated that 160-300 ml. were admixed with the maternal blood. Several additional reports by other workers have since confirmed Chown's findings.[3, 11, 13, 36]

Although Chown demonstrated that fetal cells could, indeed, pass the placenta giving rise to a gross hematological defect, several questions bearing directly on Rh sensitization in the mother have not been answered as yet. *First,* is feto-maternal erythrocyte transfer a normal event or does it represent pathology of some sort? *Second,* when do fetal cells cross over and by what route? *Third,* how much fetal blood is necessary to produce maternal immunization? *Fourth,* what other factors influence iso-immunization of the mother during pregnancy?

In answer to the first question, if feto-maternal erythrocyte transfer is not a universal phenomenon, it is, at least, quite common. Zipursky *et al.*[48] employing a technic which takes advantage of the greater solubility of adult hemoglobin in a fixed smear in acid phosphate buffer, showed that nine of 42 postpartum women had fetal cells in their circulation. Creger and Steele[7] using the Ashby technic found 10 of 14 mothers of ABO compatible babies to have fetal cells mixed with their own in the proportion of 1:800 fetal/maternal cells. (Richardson-Jones[38] estimates 1:2,000-5,000 by the mixed cell agglutination technic.) Hosoi,[15] employing differential agglutination of fetal cells with anti-ABO or anti-MN sera, detected feto-maternal mixtures in the maternal blood in ten of 24 ABO-compatible births and seven of 23 ABO-incompatible births. Dunsford[10] inferred fetal cell transfer in all of four postpartum mothers studied using a method which measured a decrease in maternal anti-A and/or anti-B titer. In one case, fetal cells could be directly observed by differential agglutination. Tests for the secretor status of the children were not reported. Cohen, Zuelzer and Evans[6] demonstrated Rh positive cells in the circulation of four of six pregnant Rh negative women by the fluorescent antibody technic. Positive results were recorded as early as six weeks prepartum. In none of these series were the babies anemic or in any other way clinically affected with a hematological disorder except in the series reported by Cohen *et al.*[6]

in which there were three cases of erythroblastosis and a stillbirth.

Rucknagel and Chernoff[39] detected fetal hemoglobin in the blood of ten of 91 women in the second trimester of pregnancy by means of a fetal hemoglobin antiserum. These authors, however, felt that the fetal hemoglobin was produced by the mother in response to the stress of pregnancy. The authors were careful to point out that their method was not sensitive enough to detect minute amounts of fetal blood which may have crossed the placental barrier.

The second question is much more difficult to answer. There is no available evidence to suggest whether tiny placental breaks do, in fact, occur naturally or as an event of parturition or both. Some reports suggest that cell transfer probably begins about the beginning of the third trimester since, in previously sensitized women, the titer begins its slow rise about that time in many cases. Possibly the greatest amount of transplacental passage occurs at birth for many investigators have noted a marked rise in titer postpartum. There has been much speculation about the relationship of the onset of Rh antibody production and the height of the maternal antibody titer to the severity of hemolytic disease. This aspect has been exhaustively reviewed by Freiesleben[12] and will not be discussed here.

The third question may be answered quite briefly. The amount of blood necessary to immunize the mother is probably very small. In cases in which the first-born has been affected, it has been determined that the mother had been previously transfused with as little as 10 ml. of whole blood. Levine[19] believes that as little as 0.067 ml. of packed fetal cells can produce immunization. Allen and Diamond[1] also concede that as little as 0.1 ml. can do so. The remainder of this review will be devoted to answering the last question.

Evidence for Actively Acquired Tolerance to Rh Antigens

Mollison, Mourant and Race[29] estimate that the risk of immunization to **Rh$_o$** (D) is approximately one in 22 in all pregnancies beyond the first. The risk is greater when the husband is homozygous (1 in 15) than if he is heterozygous (1 in 32). Various explanations have been offered to account for this low incidence including the failure of fetal cells to cross the placenta and an "innate" resistance of the antibody forming mechanism of the mother. Some workers maintain that the paternal Rh genotype is instrumental in determining the antigenicity of the **Rh$_o$** (D) factor, some being more antigenic than others. This idea has been extensively studied by Murray[30] who found that R^2r (cDE/cde) infants were more severely affected than those of other genotypes. This conclusion has been challenged by Kirk, Vos and Kelsall[18] although the latter authors recognize that sampling errors may have contributed to their discrepant results. Levine *et al.*[22] have provided circumstantial substantiation of Murray's results by demonstrating *in vitro* differences in the reactivity of the **Rh$_o$** (D) factor with saline (complete, bivalent) anti-**Rh$_o$** (D) sera. Dosage effect titrations indicated that R^2R^2 (cDE/cDE) cells were more reactive than R^1R^1 (CDe/CDe) and R^or (cDe/cde) cells. In addition, some antigens such as the **Rh$_o$** variant (D^u) are only very poorly antigenic in controlled injection studies, requiring many infusions for an antibody response if, indeed, one is obtained at all.[41, 44]

In 1954, Owen and his co-workers[34] published an interesting series of observations which seemed to indicate that an Rh negative person might be rendered immunologically tolerant to Rh antigens if at some time during intra-uterine life, Rh positive cells from the maternal circulation had, somehow, passed into the fetal bloodstream. With the aid of the Pasadena

TABLE 1. *Los Angeles Data**
(Based upon cases of erythroblastosis fetalis)

	Grandmaternal Rh Type		
	Positive	Negative	Total
Tolerant	2	0	2
Intolerant	12	7	19
Total	14	7	21

* Owen *et al.* Proc. Nat. Acad. Sci. **40:** 420, 1954.

Rh Testing Laboratory, Owen collected 51 cases of Rh negative women who had delivered at least three Rh positive children. The development of Rh antibodies had never been detected in 34 of these, whereas the remaining 17 developed Rh antibodies in three or less pregnancies. The Rh types of the grandmother, the mother, her husband and the children are known from tests at the same laboratory.

In another series collected from the Hematology Clinic of the Los Angeles Children's Hospital, Owen classified 21 Rh negative women according to the Rh type of their respective mothers (Table 1). These were then further subdivided according to whether the Rh negative mothers delivered an erythroblastotic infant during or before their third Rh positive pregnancy. The first group was classed as relatively tolerant, the second as relatively intolerant.

The Los Angeles series (Table 1) differs from the Pasadena series (Table 2) in that the latter are unselected cases classed on the basis of Rh sensitization whereas the former group was selected by the occurrence of frank hemolytic disease.

The interpretation placed on these data by Owen considers that no apparent tolerance is conferred upon an Rh negative woman whose mother is Rh positive when tolerance is based upon the appearance of erythroblastosis alone. This same conclusion was reached in other independent studies in England,[2] Australia[45] and this country.[25]

On the other hand, Owen suggests that the Rh negative daughters of Rh positive women may enjoy relative tolerance if the presence or absence of antibodies in their sera is used as the criterion of tolerance. Unfortunately, data on unselected cases necessary to confirm or refute these findings is rather difficult to obtain and is lacking in other studies. Ward *et al.*[45] were, however, able to demonstrate that the severity of the disease and the height of the maternal titer are also independent of the grandmaternal phenotype, but, again, their data are based solely on affected infants.

Evidence Against Actively Acquired Tolerance to Rh Antigens

As early as 1943, Levine[20] noted that the Rh negative mothers of erythroblastotic offspring are less frequently ABO-incompatibly mated than expected. This has

TABLE 2. *Pasadena Data**
(Based on antibody determinations only)

	Grandmaternal Rh type				
	Positive		Negative		Total
	Obs.	Exp.	Obs.	Exp.	
Tolerant	25	(20.7)	9	(13.3)	34
Intolerant	4	(10.4)	13	(6.6)	17
Total	29		22		51

$\chi^2 = 2.10$
$p = .15$ d.f. $= 1$

* Owen *et al.* Proc. Nat. Acad. Sc. **40:** 420, 1954.

TABLE 3. *Tolerance Based upon Mother-Child Incompatibility*†

	ABO-Compatible Obs.	ABO-Compatible Exp.	ABO-Incompatible Obs.	ABO-Incompatible Exp.	Total
Tolerant	18	(27.5)	16	(6.5)	34
Intolerant	13	(13.0)	3	(3.0)	16*
Total	31	(40.5)	19	(9.5)	50

* No data on one pair in the Intolerant class — $\chi^2 = 1.81$ $p = .17$ d.f. = 1

† Addendum insert in Levine. Human Biol. **30:** 14, 1958.

been confirmed by numerous investigations in which samples of Rh-sensitized mothers and their families have been compared to random expectancy or control groups (cf. 21 for a complete bibliography of these studies). Random expectancy should lead to 18-23 per cent ABO-incompatible mother-child combinations (mean about 19 per cent) yet when this is compared to the figures for ABO-incompatible matings with erythroblastotic children, only 4.4-12.6 per cent are actually found.[21] These figures are based upon 11 separate studies comprising 5,361 matings. The reasons for this wide variation are discussed by Levine.[21]

The rationale employed to explain this finding states that ABO-incompatible cells would be destroyed in the maternal circulation by the natural ABO antibodies and would thus persist for a duration insufficient, in many instances, to produce Rh sensitization. Conversely, ABO-compatible cells would not be destroyed and might circulate in the maternal system for longer periods.

In a 1958 paper, Levine[21] examined the findings of Owen *et al.* and postulated that the Pasadena data might also be explained if the tolerant class contained an excess of ABO-incompatible pregnancies and the intolerant class presented an excess of ABO-compatible matings. In a mimeographed correction sheet inserted into reprints of his paper, Levine contends that this is, indeed, so (Table 3).

It is obvious that Owen's study is necessarily a preliminary one and that the figures are too few to permit a preference of one explanation over another except by intuitive bias. If Owen is correct, then tolerance, when it is induced, is subject to subsequent breakdown and cannot, therefore, be treated as an "all or none" principle. The degree of tolerance appears to decrease as the number of pregnancies increases. Also, in considering Levine's hypothesis, although we are dealing with a well documented statistical effect, the mechanisms operative are complex and not yet fully understood. The efficiency of the mother in removing ABO-incompatible fetal cells must depend in part upon her antibody titer which varies not only among individuals but also with the season,[42] the volume of fetal cells transferred and the period of pregnancy at which the transfer is initiated. Finally, *in vivo* experiments with human volunteers[8, 43] provides clear confirmation that ABO-Rh interaction is often sufficient for suppressing the formation of Rh antibodies in Rh negative individuals. That this is the only explanation, however, must await further studies along the lines pioneered by Owen.

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Short Communications

Incidence of the Blood Group Antigen Mi^a in the Caucasian and Negro Populations of Western New York*

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The incidence of the blood group antigen Mi^a among Caucasians of Western New York was found to be one in 715; among Negroes one in about 305.

THE INVESTIGATION of a severe case of hemolytic disease of the newborn in 1951 by Levine, Stock, Kuhmichel and Bronikovsky[1] led to the discovery of the antigen Mi^a, later found to belong to the MNSs blood group system.[5] Its evidently very low incidence in the general population was indicated by the fact that no further example was detected in testing 425 randomly selected people.[1] Employing three examples of anti-Mi^a sera discovered almost simultaneously in Buffalo and in Glasgow, a total of nine positive reactors was obtained among 3,844 random white persons examined in Glasgow and in London.[3] This made it appear as if incidence of Mi (a+) might be one in 427 white people. However, it could be clearly shown[3] that the anti-Mi^a sera which were used contained two antibodies, anti-Mi^a and anti-Vw. Among the nine positives, the cells of three were agglutinated when tested with anti-Vw sera known to have but one antibody, anti-Vw. If the interpretation[3] is correct that only two antigens are needed to explain the pattern of reactions (Vw for bloods agglutinated by the antisera containing anti-Mi^a+Vw and by the monospecific anti-Vw antisera and Mi^a for bloods reacting only with the antisera containing anti-Mi^a+Vw) then, these three should not be classified as Mi (a+). Therefore, it looks as if the incidence of Mi (a+) among the Glasgow and London samples of Caucasian origin is more nearly one in 640.

The immune example of anti-Mi^a found in Buffalo[2] was used to test randomly selected blood specimens from normal blood donors and patients routinely received in the blood grouping laboratories of The Buffalo General Hospital, Veterans Administration Hospital and The Sisters of Charity Hospital, Buffalo. The potency and characteristics of the antibodies in this serum were such that a direct saline agglutination test was most satisfactory. Of a total of 3,574 unrelated persons of Caucasian ancestry tested, six were found to be positive. Since one of these reacted with anti-Vw serum and yielded negative results with a monospecific anti-Mi^a serum, only five then could really be considered Mi (a+). Thus, the frequency of Mi (a+) in this group is one in 715.

The occurrence of the Mi^a antigen in Negroes has already been alluded to.[4] In this study samples from 609 unrelated Negroes were examined. Two were found positive, giving a frequency of Mi (a+) in these Negroes of one in about 305. Both were negative when tested with anti-Vw serum.

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Independent Segregation of the Antigen Wr^a*

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HOLMAN,[2] in his original report of the antigen Wr^a, showed that it segregated independently of the ABO, MNSs, P, Rh and Kidd antigens. Its independence of Rh has since been reported by Wiener and Brancato;[4] of ABO, MN, Rh and Duffy by Kornstad;[3] of Lutheran and, without publishing the details, of MN, Rh and Duffy by Allen.[1] Allen also gave as a reference a personal communication from David Hatcher of the Houston (Texas) Antibody Club but did not state Hatcher's findings. In the family here reported Wr^a segregates independently of the ABO, MNSs, Rh, Duffy and Lutheran antigens, confirming earlier reports. (Fig. 1)

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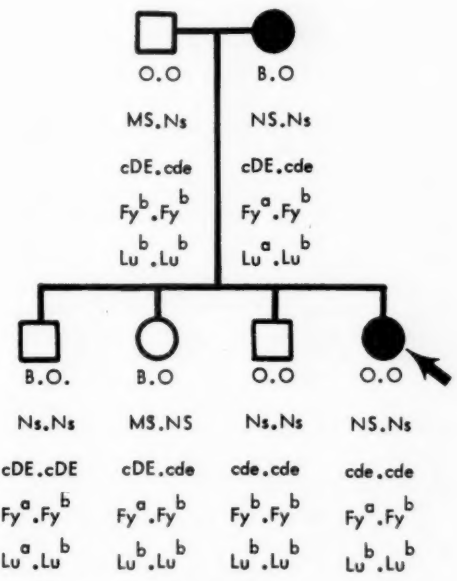

FIG. 1. Stout.

FIG. 1. Wr (a+) in Black, Wr (a—) in White. For the blood groups shown anti-A, B; M, N, S, s; CC^w, C^w, c, D, E, e, ce; Fy^a, Fy^b; Lu^a and Lu^b were used.

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Abstracts

BLOOD GROUPS AND ANTIBODIES

An Rh Gene Complex Which Produces Weak c and e Antigens in a Mother and Her Son. M. N. Metaxas and M. Metaxas-Buhler. Blutspendezentrum des SRK, Zurich, Switzerland. Vox Sang. 6: 136, 1961.

THE red blood cells of a normal donor, who was being used as a source of standard reagent cells, were noted to give reactions *in vitro* with only some anti-c sera. Weak reactions were also demonstrated for e and the propositus' mother had the same unusual cells. There was no reaction with four anti-C sera which did agglutinate r^G and r^M cells. Unlike cells with the r^G chromosome, these cells did react with anti-f. The gene complex is therefore one not previously described and the symbol r^L is suggested. —*P. J. S.*

The Diego Factor in a Puerto Rican Family: A Case of Anti-Diego. H. J. Van Peenan, J. Scudder, J. A. Jack and E. Awer. Presbyterian Hospital, New York, N. Y. Blood. 17: 457, 1961.

A PUERTO RICAN family was investigated when routine tests revealed that their premature twins were direct Coombs positive. Sensitization was due to the Diego factor: the mother was Di (a—), the father and babes Di (a+) and the maternal serum contained anti-Di^a. The twins developed no clinical evidence of hemolytic disease.

Using the mother's serum 609 other Puerto Ricans were tested for the Di^a antigen: all were negative.—*M. L.*

A Weak B Red Cell Antigen Co-existing with Serum Anti-B Antibody: Review of Literature and Investigation of a Case. D. F. Morrow, R. Hasselback and J. W. Thomas. Vancouver General Hospital, Vancouver, Canada. Canad. M. A. J. 84: 1076, 1961.

A GROUP A patient with cancer was shown to have a B-like antigen which reacted weakly with some anti-B sera but not at all with others. The patient's serum contained no anti-A but an apparently normal anti-B; she was a secretor of A substance but not of B substance. From family studies the B-like antigen was deduced to be an acquired characteristic.—*M. L.*

Maternal Sensitization Due to Bi: A Presumed "New, Private" Red Cell Antigen. M. B. Wadlington, W. H. Moore and R. C. Hartmann. Vanderbilt Univ. School of Medicine, Nashville, Tenn. Am. J. Dis. Child. 5: 623, 1961.

AN ANTIBODY defining a new antigen, tentatively named Bi, was discovered when the postpartum serum of a woman with a history of three pregnancies was found to react with her caucasian husband's cells. The antibody reacted by the indirect Coombs method only (titer 1:96).

The investigation was carried out because the patient had delivered a premature infant which developed icterus gravis followed by death at seven days. The baby had been reported to be direct Coombs negative but post mortem examination showed evidence of hemolytic disease. In the succeeding two pregnancies the patient's anti-Bi titer rose from 1:64 to 1:256 but the two resulting babes were Bi-negative, direct-Coombs-negative with no evidence of hemolytic disease. Anti-Bi was demonstrable in the cord serum in each of these.

The antigen Bi was shown to be distinct from all other known "private" or "low frequency" antigens. Investigation of the husband's family showed simple mendelian inheritance of the Bi antigen through four generations and in three branches of the family. A search for further example of the antigen was made on 1,289 random blood samples, on 140 Negro samples and on 181 Cherokee Indian samples without success.—*M. L.*

A Serum from a Group O Woman Containing an Autoagglutinin to Papainized Cells, a "Hidden" Anti-A Antibody, and a Saline Agglutinating Anti-E (Anti-rh″) Antibody. S. Whittingham, H. Lowy and P. Lind. The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia. Med. J. Aust., 1: 320, 1961.

A SERIES of experiments was carried out on a group O multipara because her serum at four months gestation contained an unusual autoagglutinin which reacted with all cells, adult or cord, but only if papainized (titer 1:128 at 37 C.). The serum also contained a normal anti-T agglutinin, a nonspecific cold agglutinin (titer 1:20) as well as anti-A and anti-B isoagglutinins (titers 1:16).

Absorption of the serum with either papainized or T-transformed group O cells reduced both the autoagglutinin and the T agglutinin to the same degree; the anti-A and anti-B titers were unaffected. However absorption with untreated group O cells resulted in a marked increase in anti-A titer (to 1:256) while the autoagglutinin, the anti-T and the anti-B were unaffected. By the 39th week of pregnancy the patient had produced an anti-E (titer 1:4). The baby was group O, E-positive, direct Coombs negative and clinically normal. The agglutinin to papainized cells was present in the cord serum in diminished titer. By three months post partum the enhancement of the anti-A titer could no longer be effected—*M. L.*

Quantitative Hemagglutination Studies in the Rh Blood Group System. I. The Assay of the Anti-D (Rh_o) Agglutinin. II. A Study of the D (Rh_o) Agglutinogen. R. Silber, M. B. Gibbs, E. F. Jahn and J. H. Akeroyd. Walter Reed Army Medical Center, Washington 12, D. C. Blood. 17: 282, 1961.

IN THE first study, the authors' employed Wilkie and Becker's hemagglutination method to determine the relative potencies of complete and incomplete isoantibodies in anti-Rh_o (D) sera. The potencies of ten different anti-Rh_o sera were compared concomitantly against a standard reference serum. The probable Rh-Hr genotype of the O donor of the test cells used in the assays was R^2R^2. Cells modified with trypsin were employed in the study of incomplete antibody.

Statistical analysis of data from the 10 assays of antisera revealed that (1) there was a linear relationship between the logarithm of antiserum concentration and the percentage of agglutinated cells expressed as probit and (2) the log-probit regression lines of the unknown and standard sera were parallel. Consequently, they were able to express the agglutinating strength of an unknown serum as a ratio of standard HD_{50}/unknown HD_{50} (potency ratio where $HD_{50} = 50$ per cent hemagglutination endpoint). Concomitant testing of the standard with an unknown serum when determining the potency ratio was found essential because significant daily fluctuations of the HD_{50} of a given serum occurred.

The average standard error of the log potency ratios of the 10 tested sera was calculated to be 9.9 per cent, with a range of 3.3 to 19.9 per cent. The relative potencies of the anti-Rh_o (D) sera were determined with an average error of 29 per cent (95 per cent confidence limits with a range of 9.3 to 52.5 per cent).

The reversibility of reaction between the Rh_o factor and anti-Rh_o was established by two independent methods.

In the second study, the same quantitative hemagglutination method was employed to compare the reactivity of Rh_o positive cells having various Rh-Hr phenotypes.

Forty-three Rh_o positive cells compared to standard cells (donor's probable genotype = R^2R^2) were tested against a single saline agglutinating anti-Rh_o serum. The log-probit regression line was found with certain cell types (probable genotypes = R^1r and R^0r) to deviate from linearity in the proximity of 50 per cent agglutination; consequently, a 30 per cent endpoint (HD_{30}) was used in comparison of these cells.

The agglutinability of Rh positive cells by saline agglutinins was found to progress in descending order of agglutinogens expressed by genes as follows: R^0 (*-D-*) > R^2 (*cDE*) > R^0 (*cDe*) > R^1 (*CDe*). Decreasing agglutinability was correlated with (1) progressively greater HD_{50} and lower slope of the log-probit progression line, (2) higher free cell counts at maximal agglutination in the midzone and (3) more pronounced tendency to exhibit prozoning.

The authors felt that they had confirmed the suppressed reactivity of the D antigen when the genes *D* and *C* are on opposite chromosomes (*trans* position) and suggested also the possibility of an inhibitory effect on the D antigen when the *C* and *D* genes are in *cis* position. They also postulated that the *e* gene in *cis* position to the *D* gene results in suppressive reactivity of the D antigen.

In a study on the effect of so-called "zygosity for the D antigen," differences in agglutination behavior between cells from homozygous and heterozygous donors were found within families. However, a marked difference was noted in one family between sister and brother of genotype, R^0r. —P. M.

An Evaluation of the Bromelin Method by Means of Comparison with the Indirect Coombs Test in Detection of Erythrocyte Antibodies. A. Claflin. Veterans Administration Hospital, Minneapolis, Minn. Am. J. Clin. Path. 35: 420, 1961.

A COMMERCIAL bromelin preparation was compared to the indirect Coombs procedure in detecting antibodies. Neither test was positive when the serums of 1,627 healthy blood donors were used. The technics were equally effective in detecting three examples of anti-Rh_o (D) and two of anti-rh′ (C). However, the bromelin method missed all the following low titer antibodies found by the indirect Coombs procedure: one anti-rh″ (E), two anti-Fy^a, three anti-K and one anti-Jk^a.—*H. A. P.*

Some Properties of Human Antibodies. J. S. Beck. University and Western Infirmary, Glasgow, Scotland. Brit. J. Exp. Path. 42: 7, 1961.

A SURVEY of human sera from normal blood donors and from hospital patients revealed that some of the samples were capable of agglutination of group O Rh positive erythrocytes previously sensitized with anti-D serum. These sera contained no irregular hemagglutinins. This anti-antibody is not neutralized by heat inactivated or untreated anti-D serum. It appears to react with antibody in an immune complex with the antigen rather than with unaltered antibody. The anti-antibody is heat stable, does not fix complement, has an optimum *p*H range of 7.5-8.0 and migrates with gamma globulin on electrophoresis. The activity of the antibody is decreased when a dialysis-labile co-factor is removed from the serum. —*L. R. G.*

Comparison of the Sensitivity to Agglutination and Haemolysis by a High-Titre Cold Antibody of the Erythrocytes of Normal Subjects and of Patients with a Variety of Blood Diseases Including Paroxysmal Nocturnal Haemoglobinuria. S. M. Lewis, J. V. Dacie and D. Tills. Postgraduate Medical School, London, England. Brit. J. Haemat. 7: 64, 1961.

A SERIES of erythrocyte samples were examined for agglutinability and sensitivity to hemolysis by serum containing high titer cold antibody. The red blood cells from patients with blood diseases showed increased agglutinability and enhanced sensitivity to hemolysis when compared with the control series which included cells from normal adults, newborn infants and patients with "medical" disorders. Blood samples from patients with paroxysmal nocturnal hemoglobinuria, megaloblastic anemia, myelosclerosis, aplastic anemia and leukemia gave the highest values for hemolysis. —*L. R. G.*

***p*H Dependent Hemolytic Systems. I. Their Relationship to Paroxysmal Nocturnal Hemoglobinuria.** S. Yachnin, M. T. Laforet and F. H. Gardner. **II. Serum Factors Involved in Red Cell Lysis.** S. Yachnin and F. H. Gardner. Harvard Med. School, Boston, Mass. Blood, 17: 83 and 474, 1961.

THE SUSCEPTIBILITY of PNH red cells to hemolysis by fresh human serum has been attributed to alterations of the stromal surface of the cell. The hemolysis can be accentuated by various acidifying agents. The hemolysis can be inhibited by heating the serum at 56° C. for 30 minutes, removing any single component of complement from the serum,

removing properdin from the serum or depleting the system of magnesium ions. Other less well known but more complex factors must also influence the PNH hemolytic system, *i.e.*, thrombin greatly enhances and dextran will inhibit the lysis of PNH cells.

Normal human red cells were treated with various proteolytic enzymes, trypsin, ficin, papain and bromelin. Other cells were treated with cholera vibrio filtrate, influenza virus and periodate ion. The treated red cells were then found to be susceptible to acid hemolysis in compatible normal human serum.

While human sera had varying capacities for PNH red cell hemolysis, no serum was studied that was devoid of lytic activity. In contrast, sera varied widely in their capacity to lyse artificially altered red cells, some sera showing no activity while others provided complete hemolysis.

Seventy-six human sera were observed for their capacity to agglutinate and/or hemolyze a panel of artificially altered red cells. Most sera showed moderate to intense hemolysis of papain, trypsin and cholera vibrio culture filtrate treated cells while only the minority of sera had this degree of hemolysis for bromelin or ficin altered red cells. The lysis of periodate treated cells was intermediate between these two groups.

No correlation was noted between the capacity for altered red cell lysis by various sera and their complement titers.

No relationship was found between the ability of serum to produce PNH red cell lysis and lysis of any of the artificially altered red cells of the panel.

The exposure of serum to altered red cells produced absorption of serum factors and impaired the lytic capacity of the serum. Cross reaction inhibition of hemolysis was observed especially by cells altered by proteolytic enzymes. Sera incapable of lysing a certain altered red cell supported the hemolysis of a coated altered red cell. The hemolysis in sera was found to be dependent upon two heat labile factors, probably hemolysin and complement. Hemagglutinins, when present were heat stable.

The various theoretic considerations of these findings are discussed and the practical importance emphasized. The fact that certain sera may contain potent agglutinins for red cells treated by the enzymes, ficin, bromelin, papain and trypsin explains the previously noted high incidence of false positive reactions in the use of enzyme treated cells for the detection of incomplete antibodies. Since sera may vary widely in their agglutinating capacity the use of a single control serum may be misleading. This hazard suggests that the antihuman globulin serum technic is still the procedure of choice for the detection of incomplete antibodies of any variety.

The relationship of moderate or overwhelming infection with pan- or poly-agglutinability of the red cells of patients with acquired hemolytic anemia is suggested by the results reported in this study. The similarity of the serum factors noted in this report to that described by Dausset, *et al.*, in a patient with acquired hemolytic anemia, and designated Tn is noted.—*A. S.*

Anti-Gamma-Globulin Consumption Test in Hashimoto's Disease. E. J. Field and A. Ridley. Royal Victoria Infirmary, Newcastle-upon-Tyne, England. J. Clin. Path. 13: 448, 1960.

THE INDIRECT antiglobulin consumption test employing patient's serum was modified for use in the diagnosis of Hashimoto's disease. The results of this method correlated well with the results of the method utilizing the tanned red cell. Positive results were obtained (a) in 16 of 18 cases of Hashimoto's disease in which circulating antibody was demonstrated with the tanned red cell test and (b) in 10 of 15 cases yielding negative tanned red cell tests.—*R. P.*

Effect of Mercaptanes in Cold Agglutinin Disease. S. E. Ritzmann and W. C. Levin. Univ. of Texas Medical Branch, Galveston, Texas. J. Lab. Clin. Med. 57: 718, 1961.

THE EFFECT of mercaptanes on the activity of cold agglutinins *in vitro* and *in vivo* was studied. The rationale was based on the known sensitivity of macroglobulins to sulfhydryl moieties. Cold agglutinins have been demonstrated to be macroglobulins. Pencillin G potassium, d-l penicillamine, cysteamine and mercapto-pyridoxine reduced cold agglutinin titers in vitro apparently by depolymerization. The administration of d-l penicillamine and mercapto-pyridoxine to a patient lowered the cold agglutinin titers and improved the Cr^{51} survival time of the erythrocytes.—*R. P.*

STORAGE AND TRANSFUSION

Collective Review. The Preservation of Whole Blood. W. F. Ballinger, II and H. E. Cohn. Jefferson Med. Coll. Philadelphia, Pa. Surg. Gynec. Obstet. 112: 411, 1961.

THIS REVIEW of blood preservation is condensed into 12 pages and 130 references are

cited. Three main subjects, anticoagulation, surfaces, and storage, are each discussed with clarity and in detail. After the evidence for and against citrate intoxication is reviewed, it is concluded that the citrate ion may be dangerous to the human recipient only under unusual circumstances. Leading articles on the use of heparin, EDTA, Edglugate Mg, CPD, glycerol and adenosine are also reviewed.

Evidence for and against the use of plastic, glass and siliconized containers plus the use of other special donor equipment is discussed. Finally, the use of refrigeration at 4 C. and at much lower temperatures, and methods to prevent homologous serum hepatitis are presented. (This is an excellent short review that should be read in its entirety by all but the most learned.—Ed.)—*J. B. R.*

Some Biochemical Changes in the Stored ACD Blood. L. Mircevova and J. Vosykova. Institute of Haematology and Blood Transfusion, Prague, Czechoslovakia. Vox Sang. 6: 179, 1961.

After demonstration that storage of ACD blood at 4 C. causes a progressive decrease in the ability of the red cells to consume oxygen (in the presence of methylene blue) and glucose, the authors focused on the less-studied pentose pathway. Results showed a progressive fall in the level of red cell pentose with storage, although there was no change in activity of transketolase or in the ability of the cells to utilize endogenous or added pentose. The authors suggest that this indicates a defect in pentose synthesis. The influence of added pentose on the viability of stored red cells deserves investigation.—*H. A. P.*

Transfusion of Postmortem Human Blood. J. Kevorkian and G. W. Bylsma. Pontiac General Hospital, Pontiac, Mich. Am. J. Clin. Path. 35: 413, 1961.

Encouraged by Russian experience, the authors transfused blood from four victims of sudden death. Volumes of blood varying from one to three pints per donor were collected into standard ACD blood bottles. The most significant abnormality in these postmortem bloods was a serum potassium varying from 13 to 24 mEq/L. The recipients had no evidence of a transfusion reaction and the expected rises in hemoglobin levels occurred.

The authors point out the following advantages of the procedure: Autopsy of the donor ensures freedom from transmissible disease to a degree greater than can be obtained with living donors. Multiple units from a single donor require only one crossmatch procedure and limit the number of red cell antigens to which the recipient is exposed.—*H. A. P.*

Profuse Bleeding Due to Transfusion of Contaminated Blood. Report of 5 Cases. L. S. Medal, R. Gonzalez C., R. Mancera and J. L. Dominguez, T. Hospital de Entermedades de la Nutricion, Mexico City, Mexico. Vox Sang. 6: 170, 1961.

In a one-month period, five patients in a single hospital received blood suspected of being contaminated. Evidence that these cases had received contaminated blood was indirect but very convincing. Poor sterilizing and storage procedures were responsible. Units of blood found infected showed no gross hemolysis.

Four of the five cases died, two of them within three hours. Massive generalized bleeding and profound hypotension were the most prominent signs. Evidence was obtained that fibrinolytic activity was responsible for the bleeding. In the most completely studied case other coagulation tests were surprisingly normal, but the serum lysed a normal clot in less than ten minutes and fibrinogen was only 117 mgm. per 100 ml.—*H. A. P.*

Measurement of Blood Volume with a New Apparatus. J. A. Williams and J. Fine. Harvard Medical School, Boston, Mass. New Engl. J. Med. 264: 842, 1961.

An ingenious device is described which makes measurement of the circulating blood volume sufficiently automatic, simple and precise that serial determinations become possible with a minimum of effort. It can be operated by a technician of ordinary competence.—*H. A. P.*

Total Red Cell Volume Changes Associated with Extensive Surgery. R. C. Hoye, A. S. Ketchan and N. I. Berlin, National Institutes of Health, Bethesda, Md. Surg. Gynec. Obstet. 112: 697, 1961.

Sixteen patients undergoing radical surgery had serial total red cell volume determinations made by the chromium-51 technic. Blood loss was usually greater than estimated by other technics. Estimation by the LeVeen automatic conductivity bridge was accurate only when the blood on surgical drapes and gowns was included (15 to 42 per cent of the blood lost). Insidious disappearance of blood postoperatively resulted in an average decrease of

22 per cent of the immediate postoperative total red cell volume. This was frequently unrecognized without blood volume determinations. There was poor correlation between changes in the total red cell volume and in the hematocrit.—*H. A. P.*

Serial Microhematocrit Determinations in Evaluating Blood Replacement. R. G. Jacobs, W. S. Howland and A. H. Goulet, Memorial Hospital, New York, N. Y. Anesthesiology. 22: 342, 1961.

SERIAL microhematocrit readings were followed in over 200 patients during various degrees of surgical hemorrhage. Examples are cited in which the changes in microhematocrit values provided information that indicated when to transfuse, how much to give, and the type of blood product required. Interpretation of the hematocrit value requires correlation with the entire clinical picture. The authors believe that serial blood volume determinations are time-consuming, costly and complicated, while microhematocrits are simple, inexpensive and accurate. The accuracy with which changes in microhematocrit reflect those in the total circulating red cell volume depends on the speed with which acute hemorrhage is followed by hemodilution. In the authors' experience, this has taken only minutes.—*H. A. P.*

ERYTHROBLASTOSIS

Isoagglutinins Associated with ABO Erythroblastosis. S. Kochwa, R. E. Rosenfield, L. Tallal and L. R. Wasserman, Mount Sinai Hospital, New York, N. Y. J. Clin. Invest. 40: 874, 1961.

THE DEVELOPMENT of a prenatal test which might anticipate the delivery of a newborn with ABO hemolytic disease was explored. Under investigation were the anti-A and anti-B agglutinins associated with the 7Sγ_2-globulins which traverse the placental barrier. The incidence of this type of isoagglutinin in type O mothers, but not in type A and B women, was consistent with the distribution of ABO erythroblastosis in the offspring of type O mothers. The 7Sγ_2-globulins as well as the associated isoagglutinins were found to be in equilibrium on both sides of the placenta. Some but not all of the 7S isoagglutinins which were difficult to inhibit with specific soluble blood group substances were associated with ABO erythroblastosis. Individuals immunized with blood group substances were also found to have type specific non-inhibitable isoagglutinins.—*R. P.*

Experimental Studies on the Prevention of Rh Haemolytic Disease. R. Finn, C. A. Clarke, W. T. A. Donohoe, R. B. McConnell, P. M. Sheppard, D. Lehane and W. Kulke. Dept. of Medicine, University of Liverpool, Liverpool, England. Brit. Med. J. 1: 1486, 1961.

PROVOCATIVE experiments to test the hypothesis that maternal sensitization to Rh positive fetal red cells might be prevented by treatment with Rh antibody are described. The presence of fetal red cells in the maternal circulation after delivery was demonstrated in 30 (11.7%) of 256 women. The ABO blood groups were tested in 135 Rh negative women of this series; in 21 cases in which fetal cells were found, the fetus was compatible with the mother and where no fetal cells were detected, the two were incompatible. Only four of the 30 women with fetal cells in their circulation had received 5 ml. or more of fetal blood. Rh antibody formation was most frequent in the women who received the larger quantities of fetal blood; only one of 75 women in whom no cells were found developed Rh antibodies. This aspect of the work was designed to establish the incidence of transplacental fetal bleeding and the correlation between circulating fetal cells and the production of Rh antibodies.

Experimental protection against Rh sensitization of the Rh negative male was investigated. Six volunteers were each given 5 ml. of Rh positive blood labeled with Cr^{51}. Thirty minutes later half of the men received 10 ml. of anti-D serum (titer 1-64). In those subjects who received anti-D, 50 per cent of the Rh positive cells disappeared within two days; the cells survived normally in the control males. The authors suggest that further study with these technics may make it possible to prevent sensitization of Rh negative women by the fetus. —*R. P.*

ABO-Incompatibility and Rh-Immunization in Pregnancy. Immunological Considerations Based on an Analysis of 148 Two-Children Families. J. Andersen, M. W. Bentzon and S. O. Larsen. Staten Serum-institut, Copenhagen, Denmark. Vox Sang. 6: 3, 1961.

THIS REPORT is an analysis of 148 two-children families in which the mother had been Rh immunized during her second pregnancy. Two groups were compared: in the first group both children were ABO compatible with the mother; in the second group the first child was compatible, the second child incompatible with

the mother. The maternal Rh titer in the last ten days before delivery and the strength of the direct Coombs test on the cord blood cells from the affected child were significantly lower in the second group.

These observations support the theory that an ABO incompatible fetus is a lesser stimulus in Rh immunization than an ABO compatible one.—*A. S.*

"Enclosed" Hemorrhage and Neonatal Jaundice. A. R. Rausen and L. K. Diamond. Children's Hospital Medical Center, Boston, Mass. Am. J. Dis. Child. 101: 164, 1961.

HEMORRHAGE into the tissues of newborn infants is discussed as a cause of hyperbilirubinemia. Three such cases are described in which the serum bilirubins rose significantly. Exchange transfusions were successful in keeping the bilirubin levels below 20 mg. per cent. All three of the infants recovered without evidence of neurologic damage.—*L. R. G.*

LEUKOCYTES

Studies on Leucocyte Iso- and Auto-Antibodies. C. P. Engelfriet and J. J. van Loghem. Netherlands Red Cross Transfusion Service, Amsterdam, Holland. Brit. J. Haemat. 7: 222, 1961.

THE AUTHORS summarize two years of work on complete and incomplete leukocyte antibodies. The incomplete form found by the antiglobulin consumption test (ACT) appeared in the sera of multitransfused patients three times as frequently as the complete antibody. The use of papain in the ACT test inhibited isoagglutination but not autoagglutination. The transfusion reactions induced with incomplete antibodies did not differ from those induced by complete antibodies. The proportion of pregnant women with leukoagglutinins was small, 15 of 838 women examined, probably because of the test cells employed. All the antibodies in the pregnant women were of the complete form. Autoantibodies for leukocytes demonstrated by the antiglobulin consumption test were found in patients with clinical DLE when the indirect LE test was negative—*R. P.*

Electrophoretic Studies of Leukocyte Antibodies. I. Evaluation of Experimentally Produced Antibodies. II. Study of Clinical States. G. D. Eng and S. L. Leiken. Children's Hospital, Washington, D. C. J. Lab. Clin. Med. 57: 894 and 899, 1961.

A METHOD for detection of leukocyte antibodies by microelectrophoresis was developed. Guinea pig leukocytes exposed to anti-guinea-pig-leukocyte-rabbit serum had a slower rate of migration than the cells exposed to rabbit serum lacking agglutinins.

The microelectrophoretic technic for the detection of leukocyte antibodies was applied to the sera of 30 normal human controls and 20 patients with various diseases. There was good correlation between this method and the agglutination technic; antibody was detected in 13 patients. The method using donor cells rather than patients' cells did not establish whether the leukocyte antibodies were involved in an autoimmune process. When leukocytes were exposed to red cell agglutinins, no change in migration was noted, thus indicating that this technic did not identify A, B or D antigens in the leukocytes.—*R. P.*

Direct Anti-Globulin-Consumption Test for Detection of Immune Antibodies. D. Nelken, J. Gurevitch, N. Gilboa-Garber. Hadassah Univ. Hosp., Jerusalem, Israel. Lancet. 1: 742, 1961.

A DIRECT anti-globulin consumption test is described for the detection of immune antibodies. The test is applicable to suspensions of washed thrombocytes, leukocytes or erythrocytes. Cell suspensions are added to anti-human globulin serum of known titer. After the mixture has been allowed to stand for 30 minutes, the supernatant serum is titrated against red blood cells coated with anti-D antibody. Reduction in titer of the anti-human globulin serum against the known sensitized cells indicates consumption of the anti-globulin by coating on the test cells.—*L. R. G.*

Statistical Analysis of a Series of 639 Patients Having Received Polytransfusions: Interpretation of the Conditions of Iso-Immunization. C. Salmon and D. Schwartz. Centre departmental de Transfusion Sanguine de la Seine, Paris, France. Rev. Hemat. 15: 162, 1960.

SEARCH for red cell, leukocyte and platelet antibodies was made on sera from patients studied regularly during transfusion therapy or chosen because they had had transfusion reactions. The frequency of anti-erythrocyte antibodies was five times greater in subjects who had anti-leukocyte or anti-platelet antibodies than in nonimmunized patients. The latter two varieties of antibodies very often appeared together, and their appearance is linked to the number of transfusions given. Anti-erythrocyte antibodies on the other hand appear with no correlation to the frequency

of transfusion. Although transfusion reactions were more frequent in patients with anti-leukocyte and anti-platelet antibodies, it was possible that this was a function of the fact that these were the patients who had received the most transfusions and not because they were immunized. Iso-immunization was rare in patients with acute leukemia and frequent in patients with cirrhosis. There is a capacity for iso-immunization peculiar to certain subjects, and among the complex factors affecting this capacity, sex appears to play a determining role.—*P. J. S.*

COAGULATION

Studies on the Coagulation of the Blood in Patients Treated with Extra-Corporeal Circulation. S. I. deVries, S. Van Creveld, P. Groen, E. Muller and M. Wettermark. Wilhelmina-Gasthuis, Amsterdam, The Netherlands. Thromb. Diath. Haemorrh. 5: 426, 1961.

THE USE of artificial heart-lung machines for extra-corporeal circulation entails problems of blood coagulation since many patients chosen for operation already have an impaired coagulation system. Moreover, the cardiac by-pass itself damages the blood, and the donor blood used in perfusion may be deficient in coagulation because of conditions due to anticoagulants and storage. The authors studied the coagulation mechanism in 21 patients subjected to extra-corporeal circulation. Blood drawn within two hours of operation was used both for transfusions and for priming the pump reservoir (ACD in the former and heparin in the latter). Blood samples were taken from the patients preoperatively, at the time of the heparin neutralization, two-three hours after perfusion and the following morning.

Preoperatively, a majority of patients showed a slight prolongation of the one stage prothrombin time, decrease in Factors V and X, decreased fibrinogen and impaired clot retraction. Eight of the 14 patients studied had abnormal fibrinolysis. After perfusion, a prolonged prothrombin time was the chief abnormality, being associated with a decrease in Factors V, VII, X and fibrinogen. Prothrombin itself was lowered in 50 per cent of patients examined and clot retraction was greatly disturbed. Moderate thrombocytopenia in the postoperative phase was of a self-limited character. Despite these clotting abnormalities, a hemorrhagic diathesis was not observed in any case during operation or in the postoperative period. The heparinized blood used in the heart-lung machine was also investigated before, during and after perfusion and exhibited varying degrees of thrombocytopenia, hemolysis, fibrinolysis and fibrinogenopenia.

The authors propose that patients selected for cardiac by-pass should be studied preoperatively for coagulation defects which can be corrected, *e.g.* fibrinogenopenia, increased fibrinolysis or hypoprothrombinemia. After perfusion, heparin neutralization should be controlled by use of thrombin times. Fibrinogen and fibrinolysis should be measured immediately postoperatively while, on the day following surgery, a more complete investigation, including platelet count, prothrombin time, fibrinogen, thrombin time, etc., should be done. The authors believe that, preoperatively, only thrombocytopenia, fibrinogenopenia and fibrinolysis constitute possible contraindications to extra-corporeal circulation. In conclusion the authors present several cases where fibrinolysis was treated preoperatively with prednisone and epsilon-amino-caproic acid.—*A. K.*

Effect of Fibrinogen, Blood Transfusion, and Epsilon-Amino-Caproic Acid in Two Patients with Fibrinolytic Activity. S. Blix. University Hospital, Rikshospital, Oslo, Norway. Acta Med. Scand. 169: 71, 1961.

TWO PATIENTS with stable fibrinolytic activity associated with giant hemangioma proved suitable for treatment with fibrinogen, whole blood, washed red cells and epsilon-amino-caproic acid. When fibrinogen was given, fibrinolytic activity increased and the fibrinogen had a diminished survival time. Whole blood, washed red cells and white cell poor blood were given to one patient on various occasions: all produced fever and increased fibrinolytic activity. In the same patients, intravenous epsilon-amino-caproic acid produced marked inhibition of fibrinolysis lasting from two to six hours but no consequent increased fibrinogen levels. The authors attribute increased fibrinolysis after fibrinogen transfusion to activation of plasminogen in the preparation and attribute the increased fibrinolysis after blood transfusion to leukocyte agglutination.—*A. K.*

Central Retinal Artery Occlusion Successfully Treated with Plasmin. S. P. Hecker and H. Zweng. Palo Alto Med. Clinic, Palo Alto, Calif. J.A.M.A. 176: 1067, 1961.

CENTRAL retinal artery thrombosis is considered an ocular emergency that usually results

in total blindness of the affected eye. Therapy consists of anticoagulants, vasodilators and stellate ganglion block. Ideal treatment would remove the mechanical obstruction to blood flow before irreversible retinal damage develops and would prevent recurrence.

A case is presented of a sixty-two-year-old, mildly diabetic male, who had progressive visual loss to the extent that he could only count fingers at a distance of one foot. On the third day of hospitalization, following failure of the above usual methods, an intravenous infusion of plasmin (thrombolysin) was started, 50,000 units hourly for six hours. The patient had no untoward effects from this therapy. Ninety minutes after the plasmin infusion was started, the patient reported improvement in his vision. In twenty-four hours, vision in the affected eye was 20/200, in three weeks 20/80; and in four months 20/40.

In an addendum to the above report, the authors state that three additional patients with retinal artery occlusion were treated with plasmin and anticoagulants. One case had complete restoration of vision, while the remaining two cases were not aided by the treatment. —*R. L.*

Thrombasthenia and Liver Disease. E. E. Mandel and J. Lazarson. Chicago Medical School, Chicago, Ill. New Engl. J. Med. 265: 56, 1961.

IMPAIRMENT of the hemostatic mechanism is common experience in active liver disease. Many reports stress the multiplicity of plasma clotting factors that may be deficient as well as the frequency of reduction in the number of platelets. The occurrence of a functional platelet defect has been largely ignored.

Thirty patients showing hepato-biliary disease were studied. All but one had active disease. Coagulation studies included a one-stage plasma prothrombin time, bleeding time, clotting time, clot retraction, platelet count, tourniquet test. and thromboplastin generation test. Of sixteen patients suffering from chronic liver disease associated with alcoholism, nine were thrombocytopenic. Twelve showed defective thromboplastic activity. Of six non-alcoholic patients with cirrhosis, four had low platelet counts, three of whom showed defective platelet activity. Of five cases with acute and subacute hepatic functional injury, four had functional platelet defects. Of three patients in the category of biliary disease, one had a significant defect in platelet function. Of the thirty cases studied, twenty-one were shown to have platelet functional defect.—*R. C.*

Development of Increased Factor VII Activity During the Spontaneous Coagulation of Blood. C. J. Johnson and P. F. Hjort. University Hospital Rikshospital, Oslo, Norway. J. Clin. Invest. 40: 743, 1961.

DURING normal spontaneous blood coagulation in the absence of tissue thromboplastin, Factor VII (proconvertin) activity increases 2.5 to 3 times. The authors studied the factors necessary for such Factor VII activity by assaying increases in Factor VII during the clotting of blood from patients with various coagulation disorders. From such assays, it appeared that enhanced Factor VII activity is not due to direct glass activation and that platelets, Factor V and Factor X are not required. The authors conclude that the interaction of Hageman factor, PTA, Factors VIII and IX and calcium are necessary for the development of increased Factor VII activity.—*A. K.*

Incidence of Various Coagulation Defects and Their Association with Different Diseases. H. A. Wurzel. Univ. of Penn. Hosp., Philadelphia, Pa. Am. J. Med. Sci. 241: 625, 1961.

IN A PERIOD of six years, 3,342 patients were studied for coagulation defects because of history of bleeding, present bleeding, imminent surgery or to see if a coagulation defect was present as part of another disease process. Routine coagulation tests included Lee-White clotting time, prothrombin consumption, platelet count, recalcification time, clot retraction, one stage prothrombin test and measurement of Factors V and VII. Thromboplastin generation tests and fibrinogen determinations were done when required for delineation of the coagulation defect. Thirty per cent of those studied had normal coagulograms. Thrombocytopenia was the most frequent abnormality found and was frequently associated with rheumatic and congenital heart disease. Next to the thrombocytopenia, combined defects of the clotting mechanism secondary to liver disease were most commonly observed. Hemophilia was found in 2.8 per cent of all patients studied while idiopathic thrombocytopenic purpura was seen in 1.9 per cent. Only one patient with congenital Factor V and one with congenital Factor VII deficiency were found in this series and no congenital deficiencies of fibrinogen, prothrombin or Stuart factor were encountered.—*A. K.*

Book Reviews

Experimental Immunochemistry. Ed. 2. Elvin A. Kabat and Manford A. Mayer. Springfield, Ill., Charles C Thomas, 1961, 920 pp. $26.50.

IN THE 12 years since the appearance of the first edition of this book, it has become universally accepted as the classic text in immunochemistry. The earlier edition emphasized quantitative methodology and quantitative reasoning in immunochemistry. The current volume retains these excellent features but adds an orientation based on the recent immunochemical advances which have provided and approached the elucidation of the structure of antigenic groups and of antibody combining sites.

Sections dealing with the modern chromatographic, electrophoretic, and analytical chemical techniques which have been responsible for the enormous progress in this field in the past few years have been included and the advantages and limitations of the various methods have been critically evaluated. The importance of purity of the materials under investigation is emphasized throughout.

In summary, this long-awaited second edition is a "must" for every worker in immunology.—*Hugh Fudenberg.*

The Red Cell: An Account of Its Chemical Physiology and Pathology. T. A. J. Prankerd. Springfield, Ill., Charles C Thomas, 1961, 184 pp. $7.50.

IN THIS ERA of public drives on cancer and leukemia a book dealing exclusively with the red cell is most welcome. Though the erythrocyte has received scant attention on Madison Avenue, publications concerning it are numerous and this concise monograph is timely and desirable. The organization of the material is logical and it is well presented. Morphology, aging, structure, permeability, metabolism, storage, hemolytic mechanisms and even the red cells of *other* species are mentioned. The reviewer found the chapters entitled "Molecular Structure" and "Metabolism" most absorbing. We feel obliged to mention a number of points with which we take issue. On page 111 the statement that in the hemoglobinopathies cell survival is *almost always* reduced cannot be supported when only the trait is present. In hereditary spherocytosis the reduced cell diameter is not usually associated with a decreased corpuscular volume whereas in thalassemia, which is not mentioned under "Microcytes" on page 113, microcytosis is characteristic. On page 131 the sentence, "There would appear to be pairs of genes involved in the transmission of an individual's hemoglobin make-up, so that an individual may inherit one or two abnormal genes and thus be either a hetero- or homozygote" leaves one perplexed. Consideration of thalassemia as a recessive disorder is no longer acceptable (p. 137). These criticisms and the speckling with rather frequent typographical errors and misspelled names do not seriously detract from the intrinsic value of this volume. It is recommended to clinicians and laboratory workers and will serve as an excellent springboard for anyone new to the subject. The references are ample and well chosen.—*T. J. Greenwalt, M.D.*

Questions and Answers

Anti-A in Plasma

Q. "Infant P. was admitted to our hospital at the age of six days. He was transfused at once with 75 ml. of pooled plasma, this being followed immediately by a like amount of whole blood. Five days later similar transfusions of plasma and whole blood were given. Ten days after admission, when a third crossmatch was requested, the infant was found to have anti-A_1 acting strongly in saline and albumin and by indirect Coombs. The direct Coombs also had become weakly positive. The infant typed as A_2 and now crossmatched compatibly only with donor bloods of this sub-type. A titration of the anti-A in the pooled plasma gave positive results by indirect Coombs at a dilution of 1:256. This incident prompts us to ask: may transfusions of commercial pooled plasma be given to infants and small children with the same assurance as to other patients? Do producers of pooled plasma adhere to any informal standards as to antibody content of the plasma?"—*M. T., Chicago, Ill.*

A. "Commercial pooled plasma may contain surprisingly strong anti-A antibodies, usually of hyperimmune type and therefore frequently showing anti-A_1 specificity and not being susceptible to neutralization with specific soluble A substance. The administration of pooled plasma in large amounts may result in hemolytic anemias in recipients of susceptible blood groups. In the example given in the query, 75 cc. of pooled plasma was administered twice to a newborn infant. This is a relatively large volume amounting each time to approximately 1/5 of the total blood volume. Except in dire emergencies, group specific plasma should always be used in infancy and early childhood." —*Wolf W. Zuelzer, M.D.*

Gel in Plasma

Q. "For some time we have been separating plasma from our bottles of outdated blood for the purpose of using the plasma in our own hospital. Now it is noted that some bottles of plasma develop a very soft jell-like mass at the base of the airway, sometimes occupying the total volume of the plasma. This formation develops after the six month waiting period, but at just what time has not been ascertained. However, it usually forms in the older units, though not all of the older units are affected. Cultures in thioglycollate have not revealed growth of any kind. Smears of this substance appear like fibrin. How can we prevent this formation?

The citrated whole blood we separate has 110 ml. of anticoagulant acid citrate dextrose solution B (N. I. H. Sol. B) to every 440 ml. of blood. Plasma is withdrawn with Abbott's disposable plasma aspirating set #4487 into Abbott's evacuated empty plasma container #4221. We do not pool the plasma. The plasma bottles are examined a couple of days later for presence of red cells and if any are present, these bottles are aspirated a second time. All plasma is cultured at time of withdrawal. Plasma is stored at room temperature and not used until after six months has elapsed. Storage at room temperature is continued through life of the plasma."—*M. T., Lincoln, Nebraska.*

A. "The formation of the soft jell-like fibrin mass in plasma can be prevented by using 250 ml. plasma containers containing 25 ml. of 50 per cent dextrose solution. When the plasma is withdrawn it must be thoroughly mixed with the dextrose solution. Dextrose solution has been found to have a stabilizing effect on fibrinogen.

Plasma containers with the 50 per cent dextrose solution are commercially available."—*Morten Grove-Rasmussen, M.D.*

Single Donor Plasma

Q. "I would appreciate your answer to the following questions. In our small laboratory we process about 1,000 units of blood a year for transfusion, of these perhaps 40 or 50 are administered as packed red cells. The plasma is removed by a closed system into a sterile vacuum bottle, a commercial evacuated plasma bottle being used. Could you advise me as to the advisability of saving this plasma for transfusion and also your recommendations as to the exact procedure including sterility check and storage."—*M. D., East Stroudsburg, Pennsylvania.*

A. "Plasma removed from whole blood is of much use if there is a demand for plasma in a hospital. The amount of this plasma available may, of course, be greatly increased by staff education in the use of packed cells. This aspect of transfusion therapy is fully covered in two articles entitled 'Transfusion Therapy,' appearing in the *New England Journal of Medicine* for May 18 and May 25, 1961 by Dr. Grove-Rasmussen and others.

Plasma removed by a closed system under sterile conditions into a sterile vacuum bottle should be prepared and stored in individual units, that is, as single donor plasma. This plasma should be labeled to show the ABO group and Rh type of the donor blood from which it was obtained. At the time of aspiration, a culture should be taken from the original blood container as the last portion of the plasma aspiration. Five to 10 ml. of this plasma should be placed in 10 times its volume of N. I. H. thioglycollate broth and cultured for seven days at 30 C. to 32 C., or at both room temperature (20 C. to 25 C.) and at 37 C. for the same period. Subculturing may be done in the same medium and at the same temperatures before the end of the original culture period. The original plasma aspirated into the sterile vacuum bottle should be frozen and stored at −18 C. (0 F.) or lower, and thawed in a 37 C. water bath when needed.

The above plan is the best and safest plan of plasma processing for a small laboratory utilizing bottled blood. Other processing methods and storage temperatures may be utilized by the small laboratory if the blood is in a plastic container which allows sampling without opening or venting the final plasma container."—*Mark Falcon Lesses, M.D.*

Crossmatch for Multiple Transfusions

Q. "How many pints of donor blood may be safely given with a single collection from a patient? I was always trained that not more than three pints could be given on one collection and then another sample is drawn from the patient for any further crossmatches for transfusions."—*M. T., Fort Wayne, Indiana.*

A. "The amount of donor blood which may be safely given to a patient on the basis of a single major crossmatch would depend upon the volume of blood given in relation to the blood volume of the patient, and upon the time interval between each transfusion. Thus, a pint of blood given every day for three days would require a crossmatch on each day since each pint of blood can be an antigenic stimulus. Thus, the 'Standards' of the American Association of Blood Banks state, 'When a series of transfusions are to be given over a period of days, it is essential that a new sample of the recipient serum be obtained on the day of each transfusion so that atypical agglutinins which may have resulted from the previous transfusions may be detected by the compatibility procedures.'

On a given day, many more than three pints of blood could be given on the basis of one crossmatch. Problems of compatibility between various units of donor blood given at one time do not arise until the amount of donor blood begins to approach the blood volume of the recipient. Under these conditions an intercrossmatch, or donor blood screening, or both, become necessary. As a matter of convenience, and rough approximation to the facts one may assume the blood volume to be 7 per cent of the body weight. Thus, an 80 kilo person would have a blood volume of 5,600 ml. or a little over 11 pints. In such a person it would be safe to crossmatch and infuse in a given day, on the basis of that crossmatch, considerably more than three pints."—*Mark Falcon Lesses, M.D.*

Correspondence

To the Editor (July 18, 1961):

Minus-Minus Phenotypes

"Dr. Allen in his recent editorial (TRANSF. July-August issue) discusses three possible genetic explanations for the minus-minus phenotypes, namely, deletion, recessive modifiers and amorphs. I should like to add some additional alternative explanations as well as comment on those mentioned in the editorial.

Suppressors and modifiers of gene action have been known for many years in Drosophila and elsewhere. It should, therefore, have been no surprise to find that such modifiers also exist for the various blood group genes. The studies of Levine and others have confirmed the existence of an epistatic locus, *Xx*, capable in homozygous dose of acting as a suppressor of the ABH substances of the red blood cell envelope and the secretions. Weiner has also adduced evidence of another locus, *Yy*, that modifies the expression of the A antigen on the red blood cell but with no effect on the secreted substances. This too is a recessive gene.

The pedigree of the Cr. family published by Crawford, *et al.* in the same issue is entirely consistent with the hypothesis that a dominant suppressor capable of acting in single dose may be epistatic to the Lutheran blood group system. Dominant suppressors are also found in other species.

In organisms in which it is possible to analyze suspected deletions, it is well known that homozygous deletions are almost invariably lethal. Green has shown that three loci in Drosophila once believed to be examples of homozygous deletions cannot be so explained for he has detected occasional back mutations to wild type and intermediate alleles. One should, therefore, be extremely cautious about invoking a deletion as an explanation of a viable mutant.

The term "amorph" was originally coined by Muller for mutant genes whose action was so weak or even undetectable that dosage was ineffective in producing a change in their expression. Amorphs were never considered to be genes lost by deletion.

Many of the phenotypes categorized by Dr. Allen may be due to any one or even all of the explanations given. For example, the —D— and --- phenotypes could equally well arise from the action of suppressors, as a result of deletion or through the presence of amorphs. Although isoallelism has not been demonstrated in human blood group systems, it is well to recall that identical phenotypes often arise from quite diverse causes. Clearly, it is

somewhat premature to classify the presently known minus-minus phenotypes according to genetic interpretations when it is not yet possible to distinguish between them."—JOEL M. SOLOMON.

To the Editor (July 20, 1961):

Minor Crossmatch

"I have just finished reading the panel discussion on the minor crossmatch in the current issue (July-August) of TRANSFUSION. It vividly brings to mind an instance that occurred about the time you were here. I was called to the blood grouping laboratory because all ten units of blood being crossmatched for by-pass surgery were found to be incompatible on the minor crossmatch! This was the first time in over 20,000 minor crossmatch tests that such an incompatibility had been encountered. The patient was a baby four months old and the original grouping had been performed two months earlier (Group O (patient actually Group A)). The grouping error could have been caused by lack of avidity of the infant's erythrocytes but was more likely due to obtaining blood from the wrong patient. Were it not for the minor crossmatch I am certain that the result would have been fatal. This emphasizes that the minor crossmatch for by-pass surgery is an absolute MUST."—DON R. MATHIESON, M.D., *Rochester, Minnesota.*

To the Editor (July 24, 1961):

Questions and Answers

"I was most interested in two of the answers given to questions in your May/June issue and if I may, I would like to comment briefly on the answer by Dr. Dunsford first (page 205) on the question of routine screening of irregular antibodies in blood donors. I fully agree with Dr. Dunsford on the two reasons which he gives why this investigation should be done but I feel that he has omitted a third which to my mind is at least as, and possibly more important, than the two which he lists.

It is to my mind of extreme importance to know the presence of any potentially dangerous antibodies in blood which is being transfused if the patient carries the homologous antigen. It is generally stated or thought that the dilution of such an antibody in the patient's serum is sufficient to counteract any ill effects. This, however, is certainly not so if the blood is to be given to, for instance, babies and I cannot think of a much worse procedure than to transfuse an antirhesus antibody occurring in a rhesus negative donor—and there are many amongst our donors who are immunised—into a baby suffering from haemolytic disease of the newborn due to anti-D. Again, in procedures like dialysis in an artificial kidney, the presence of an antibody acting against an antigen on other donor's cells might not be in material. In fact, it is my considered opinion that the search for antibodies in donors' blood replaces the so-called minor cross-match which is still thought essential in some laboratories (though not in mine).

As to the second question, the question dealing with erythroblastosis on page 206, I fully agree with your answer and I too am convinced that the rhesus type of the child is not the most important question. After all, a baby of rhesus type CDe/cde can be ill with haemolytic disease of the newborn due to anti-D or anti-c. It is, therefore, imperative that the serology of the mother be known for the proper provision of blood for an exchange transfusion and ideally this should be known before the baby is born. On the other hand, if ante-natal tests for some reason have not been performed, it is very easy indeed to prepare rapidly, an eluate which can be used for crossmatching in the same way as the maternal serum. This eluate, by the way, indicates with certainty whether the baby is D positive as an antibody of anti-D specificity can, of course, only attach itself to the cells if the cell itself is D positive and the same applies to antibodies and antigens other than the one mentioned.

As to the question of rhesus typing in babies giving a strongly positive direct Coombs test, it is, of course, essential to use only saline agglutinating antibodies and the cells suspended in saline and if an antibody having a high titre is used, it is in my experience very rare indeed to obtain a completely negative result, even with heavily coated D positive cells."—DR. W. WEINER, *Director Regional Blood Transfusion Service, Birmingham 15, Great Britain.*

Meeting Notes

American Federation for Clinical Research, American Society for Clinical Investigation, Association of American Physicians

April 30 - May 2, 1961

ASTER *et al.* demonstrated complement-fixing antibodies in the sera of two mothers of infants with neo-natal purpura (1 amegakaryocytic). The high titer C′-fixing antibody could not be confidently demonstrated by the other methods used. The specificity of the antibodies coincided and reactions were obtained with the platelets of the fathers and infants (after recovery) and 27 per cent of normal individuals.

Des Prez *et al.* observed clumping and fusion of platelets, transfer of 5-hydroxytryptamine and a bactericidin against *B. subtilis* when platelet-rich rabbit plasma was incubated at 37 C. with E. coli endotoxin. A heat labile plasma factor is necessary to produce these results which are markedly influenced by temperature and the nature of the anticoagulant used.

Perillie *et al.* used the skin window technic of Rebuck to demonstrate significant delay and dimunition of the early granulocytic response to local injury in diabetic acidosis.

Murray and his co-workers produced normovolemic anemia and polycythemia in dogs and demonstrated that optimum systemic oxygen transport occurs at normal hematocrit. Less oxygen is delivered to the tissues when the hematocrit deviates in either direction.

Henkin and associates found that the toxic effect of six uremic sera on HeLa cell cultures disappeared post-dialysis and appeared in the dialysate. They state that the responsible substance is a thermostable small protein molecule which migrates with the albumin and prealbumin fractions.

Jackson *et al.*, demonstrated that trypsin treated platelets suspended in serum did not clump on the addition of thrombin until fibrinogen was added. Trypsin apparently removes the fibrinogen coating of platelets without altering their morphology. It is felt that fibrinogen is the substrate of thrombin in producing platelet aggregation and initiating clot retraction.

Henderson and Rapaport discussed a powerful coagulant activity, activation product (AP), which arises when normal plasma or serum is exposed to glass or celite. Hageman factor or plasma thromboplastin antecedent deficient sera cannot form AP.

Greenough and Thomas have demonstrated a heat stable nonheme protein which enhances the uptake of Fe^{59} into hemoglobin during incubation of marrow *in vitro*. It may be related to ferritin.

In the phenomenon of immune adherence (IA) an antigen adheres to an indicator (red cells or guinea pig platelets) only in the presence of complement and specific antibody. Positive reactions were obtained when eluates and sera from patients with auto-immune hemolytic anemia (AHA) were reacted with the leukocytes from chronic lymphocytic leukemia. Brody suggests that this means that the pathogenesis of AHA is not related to the erythrocytes but rather to abnormal antibody synthesis by leukemic cells.

Marks, Gross and Banks presented evidence for the heterogeneity of the glucose-6-phosphate dehydrogenase isolated from the red cells of different examples of deficiency of this enzyme. In three members of an Italian family the G6PD had faster electrophoretic mobility and decreased affinity for DPN than the normal enzymes.

Jacob and Jandl used p-hydroxymercuribenzoate (PHMB) and N-ethylmaleimide (NEM) to inhibit sulfhydryl (SH) groups of red cells. Treatment of erythrocytes with these compounds resulted in their sequestration *in vivo* and did not appear to be dependent upon the levels of intracellular thiols such as glutathione. It was suggested that failure of mechanisms maintaining membrane thiols is important in the destruction of erythrocytes.

Ways, Reed and Hanahan studied the erythrocytes of three patients with acanthocytosis, a hereditary syndrome manifested by "spiny" erythrocytes, steatorrhea, neurologic disease and retinitis pigmentosa. The lecithin fraction of the red cell stroma was found reduced and the linoleic acid content was depressed. It now seems that these changes may be secondary to the faulty fat absorption.

Freireich *et al.*, administered repeated platelet transfusions obtained from single donors by plasmapheresis to 28 children with acute leukemia without observing any diminution in the

clinical response or the post-transfusion circulating platelet levels.

Jandl and Katz have shown that Fe^{59} labeled transferrin attaches to receptor sites on reticulocytes making the iron available for Hb synthesis. Controlled treatment of the red cells with trypsin destroys the receptor sites and blocks the incorporation of the labeled iron.

Weisberger presented a fascinating experiment which suggests the transformation of hemoglobin synthesis by erythroid precursors *in vitro*. He incubated DNA from sickle cell normoblasts with marrow from patients with pernicious anemia and after 24 hours found a fraction with an electrophoretic mobility resembling S hemoglobin but not confirmed to be S hemoglobin. These are very preliminary observations not as yet adequately controlled.

Valentine *et al.* demonstrated pyruvic kinase to be deficient in three related subjects with non-spherocytic congenital hemolytic anemia. —*T. J. Greenwalt, M.D.*

News and Announcements

FRANK E. TROBAUGH, JR., M.D., *Editor*
Chicago, Illinois

LOIS J. JAMES
Chicago, Illinois

Blood Insurance Conference

On July 29, 1961, the executive committee of the board of directors of the AABB conducted a day long conference on blood insurance programs with the payment of blood or money as the premium. Nine blood banks which have been engaged in this type of program from one to eight years were invited to participate.

Representatives from the blood bank of Hawaii, Chicago Blood Donor Service, Southwest Blood Banks, Bergen Community Blood Bank, New Orleans Blood Bank, Belle Bonfils Memorial Blood Bank, Delta Blood Bank, Minneapolis War Memorial Blood Bank and Central Iowa Blood Donor Service were present and participated in the discussion.

Each blood bank representative was asked to present an outline of his own program and make any comments based on his experience. These various programs covered groups of a few hundred to 250,000 individual subscribers. The premium for unlimited blood coverage for a period of one to two years for an individual varied from $1 to $15, with the premium for a family ranging from $2 to $15. A 90-day waiting period and a disease exclusion clause were usually parts of the insurance contracts. One of the blood banks engaged in this type of insurance program has been converted into an insurance company. Such a conversion requires a capital outlay of $100,000 or more depending upon the state in which the company is incorporated.

There was a discussion of such matters as optimum coverage, restrictive clauses, waiting period, cost analyses, loss ratios, cost of paid donors, the optimum level of the premium rate which would create a suitable balance between the number of premium payments by blood and by money, growth of insurance plans, and minimal number of subscribers necessary to provide this type of blood insurance to a community. All who were present agreed that the conference was worthwhile. This was only the beginning of the explorations which the AABB plans to undertake in its evaluation of the recruitment and procurement plans which ultimately will be considered best to meet the blood needs of the nation.—J. R. SCHENKEN, M.D.

Blood Banks Celebrate Twentieth Anniversary

In June, the Irwin Memorial Blood Bank of the San Francisco Medical Society observed its twentieth anniversary of community service. The bank, which was the first medically-sponsored, nonprofit community blood bank in the United States, has made many technical, scientific and administrative contributions. It processes over 5,000 pints of blood a month, and serves a population of 1,200,000 people through 57 hospitals in eight northern California counties. Besides its headquarters in San Francisco, the blood bank operates blood centers in Redding and in San Rafael, California.

The need for whole blood and blood derivatives is constantly increasing, according to Dr. C. Y. Gates, President of the Blood Bank Commission, and the blood bank must once again expand its quarters. Architectural plans to enlarge the bank are under way.

More than 850,000 pints of blood processed by the bank since its inception and largely contributed by volunteer donors, represents a savings of millions of dollars for patients who have received blood from this bank.

In September 1961 the John Elliott Blood

Bank of Dade County celebrated the completion of 20 years of uninterrupted public service. During this time, the Blood Bank has grown from a single room with three employees who prepared 3,600 transfusions to a large building housing 62 full time employees responsible for the preparation, last year, of 42,804 transfusions. Blood collection is carried out both at the Central Blood Bank, by mobile units and permanent branch bleeding stations.

The John Elliott Blood Bank provides a complete community transfusion service, being responsible for the blood inventory of many hospital blood banks in the area and providing substantial support to independent hospital blood banks. About 60 per cent of all the blood issued by the Bank is crossmatched on the premises, thereby providing a wealth of material for investigational and training purposes. All blood is donated voluntarily by individuals and by members of the 1,019 donor clubs in the area.

Chicago Honors World's First Blood Bank

On September 7, 1961, the American Association and the Illinois Association of Blood Banks honored Cook County Hospital and the late Dr. Bernard Fantus for the establishment of the world's first blood bank 24 years ago. At a luncheon held at the Sheraton-Blackstone Hotel in Chicago, the American and Illinois Associations, along with the 55 Chicago Community Blood Banks paid tribute to this pioneering achievement by presenting a plaque to Cook County Hospital.

Dr. Israel Davidsohn, past President of the American Association of Blood Banks, and Scientific Director of the Mount Sinai Medical Research Foundation, served as master of ceremonies for the luncheon, which also launched "Know Your Blood Bank Days in Chicago." Dr. Davidsohn said: "Chicago still is a leader in the nation in advances on blood banking and its application." Dr. Davidsohn read from a letter from Dr. Elizabeth H. Schirmer (Portland, Oregon) who was the first Director (under Dr. Fantus) of the Cook County Hospital Blood Bank:

> "Much has happened since those early days back in 1937—when our Dr. Fantus after years of effort, convinced the County Commissioners that lives would be saved at County Hospital if blood were more easily available. I remember going over charts, and one intern or resident had written a progress note which read 'Tested 21 donors—all N.G.—The family is exhausted, and so am I.' Ergo—no transfusion for that patient. As a matter of fact, before the blood preservation laboratory was functioning, an average of 64 transfusions a month were given at the hospital."

Dr. John R. Schenken, President of the American Association of Blood Banks and principal speaker, said:

> "The modern techniques of blood banking were pioneered in Chicago with the establishment of the world's first blood bank at Cook County Hospital. Since that beginning 24 years ago, the blood bank program in this community has continued to be led by men of extraordinary vision who are constantly developing and researching new techniques in blood banking.
>
> "The giving of a pint of blood is the most personal gift one can make to his fellow man. At the time Dr. Fantus established the first blood bank there were only four types of blood known. Thanks to modern blood banking which has resulted from pioneering here, bloods of more than 32 types are available to the patient in minutes.
>
> "Blood banks throughout the United States and the entire world have greatly benefited from Chicago's leadership in the field."

During the luncheon, a plaque honoring the establishment of the world's first blood bank and its founder, Dr. Bernard Fantus, was accepted by Dr. Karl Mayer, Medical Superintendent, Cook County Institutions. The plaque, two feet wide and three feet high, is inscribed: "In recognition of the world's first blood bank—1937. Since this pioneering achievement, Chicago has become a center of research in the life-saving techniques of transfusion therapy. This program, initiated and developed in Chicago, continues to benefit the people of this community, this nation, and all humanity."

Dr. John Maloney (Bloomington, Illinois), President of the Illinois Association of Blood Banks, presented the plaque in behalf of the American and Illinois Associations of Blood Banks to Dr. Meyer. In accepting the plaque, Dr. Meyer said: "Although his achievements in the field of blood banking were monumental, Dr. Fantus' influence has been felt by the entire medical world. He was a man dedicated to the alleviation of illness through all methods of medicine that would benefit a patient."

Speaking for Chicago's Mayor Richard J. Daley who proclaimed the period beginning September 7 "Know Your Blood Bank Days in Chicago," Dr. Samuel L. Andelman, Chicago Health Commissioner, said:

> "We are fortunate to be rich in medical care and health resources in Chicago. One of the most valuable of these facilities is our community blood program. Its vital importance is reaffirmed each day in terms of lives saved and health restored, and its progress here has made the city outstanding in the nation, if not the world."

Mr. Jerome Huppert, Commissioner on the Cook County Board and Chairman of the Hos-

Left to right are Dr. Karl Meyer, medical superintendent of Cook County institutions, Dr. John Maloney (Bloomington, Ill.), president of the Illinois Association of Blood Banks, Dr. John R. Schenken (Omaha, Nebraska), president of the American Association of Blood Banks, Mr. Jerome Huppert, member of the Cook County Board of Commissioners and chairman of the hospital committee, Dr. Samuel Andelman, Chicago's commissioner of health and Dr. Israel Davidsohn, scientific director of Mount Sinai Medical Research Foundation.

pital Committee, spoke on behalf of the County Board's President, Mr. John Duffy.

The period September 7-15, 1961, was proclaimed by Chicago Mayor Richard J. Daley as "Know Your Blood Bank Days in Chicago." His proclamation read as follows:

> WHEREAS, the life saving effects of human whole blood have been recognized by the medical profession and the public; and
>
> WHEREAS, the world's first modern blood bank was established at Cook County Hospital on March 15, 1937; and
>
> WHEREAS, the City of Chicago and Cook County have been the pioneering center in the development and research of blood and modern blood banking; and
>
> WHEREAS, from these pioneer accomplishments Chicago now has one of the finest community blood banking programs in the nation, with service for the public 24-hours a day, seven days a week and continually offering all the advancements of research for the benefit of all humanity:
>
> NOW, THEREFORE, I, Richard J. Daley, Mayor of the City of Chicago, do hereby proclaim September 7 to September 15, 1961, as "KNOW YOUR BLOOD BANK DAYS IN CHICAGO," and urge all the citizens of our city to acquaint themselves with the services available to them by the blood banking programs in Chicago.
>
> Dated this 29th day of August, A. D., 1961.

Michigan Association of Blood Banks Holds Two-Day Workshop in Conjunction With Annual Meeting

The Seventh Annual Scientific Meeting of the Michigan Association of Blood Banks was held on October 21, 1961 in the auditorium of the David Whitney House, Wayne County Medical Society, Detroit, Michigan.

The scientific sessions were moderated by Dr. Wolf W. Zuelzer, Chairman of the Scientific Program Committee, and Dr. Julius Rutzky, President-Elect of the Michigan Association of Blood Banks. The speakers and their topics at this excellent and stimulating program were:

Frank R. Ellis, M.D. ..... *Welcome Address*
President, Michigan Association of Blood Banks

William Turville, M.D. ..... *Long Term Blood Preservation—A Reality*
U. S. Naval Hospital

Armand J. Quick, M.D. ..... *The Relation of the Blood Bank to the Treatment of Hemorrhagic States*
Marquette University School of Medicine, Milwaukee, Wisconsin

Eric E. Muirhead, M.D. ..... *Hemolysis in Experimental Uremia Determined by Red Cell Survival*
Woman's Hospital, Detroit, Michigan

Eloise R. Giblett, M.D. ..... *Plasma Protein Polymorphisms: A New Perspective in Blood Grouping*
King County Central Blood Bank, Seattle, Washington

Flossie Cohen, M.D. and Wolf W. Zuelzer, M. D. .. *Data Pertaining to the Presence and Significance of Fetal Erythrocytes in Maternal Circulation*
Child Research Center, Children's Hospital of Michigan, Detroit, Michigan

Fred H. Allen, Jr., M.D. ..... *Management of Erythroblastosis Fetalis with Special Emphasis on Diagnosis and Crossmatching*
Blood Grouping Laboratory, Boston, Massachusetts

Frank R. Ellis, M.D. ..... *Quantitative Measurement of the Antigen in Group A Blood*
Wayne County General Hospital, Eloise, Michigan

On October 19 and 20, 1961, preceding the Scientific Session, their Sixth Annual Workshop, a two day affair, was held at Wayne State University, School of Medicine, Detroit, Mich.

New Requirement for NIH Licensed Blood Banks

"On March 7, 1961, all licensed establishments were notified of amendments to the labeling requirements of the Regulations for enforcing the Federal Food, Drug and Cosmetic Act. Most of the provisions of these amendments have been followed for all biological products except for Whole Blood (Human), Single Donor Plasma (Human) and Packed Red Blood Cells (Human) and the only change that is required for these three products is the use of a circular to provide 'adequate instructions for use.' The long established practice of providing a circular for most biological products has thus become mandatory for all products.

"All establishments having a U. S. Government license for Whole Blood (Human) were sent brief guidelines for preparing the circular on June 16. It should be emphasized that the Act for the regulation of biological products, the Regulations and the license apply only to establishments that make products that are to be shipped from one state to another for purposes of sale, barter or exchange."

Standards Committee Keeping Busy Under Dr. Lesses

On July 28, 1961, Drs. Mark Lesses and Eric Muirhead, Chairmen respectively of the Standards Committee of the AABB and the Scientific Committee of the JBC met in Chicago with other representatives from each committee. Significant revisions of the "Standards for a Blood Transfusion Service" were discussed, particularly with regard to sterility testing and compatibility testing. Important changes will be recommended shortly to the full committees of both organizations concerning these items.

Also considered at this meeting was a new and more complete form and guide for the investigation of transfusion reactions which will be submitted to the full committees for consideration.

In the AABB there has been renewed interest expressed in trying to clarify sound criteria for blood transfusions. Dr. Mark F. Lesses and Dr. Eric Muirhead have held preliminary discussions concerning this basic problem. It is hoped that their combined committees will be able to cooperate in producing a document concerning "Criteria for Blood Transfusions" which will be to blood transfusion what "Standards for a Blood Transfusion Service" is to transfusion services.

In addition to its many other activities, the Standards Committee of AABB is preparing an information circular for blood transfusions in accordance with the recent requirements of the FDA and NIH.

New Head for Peninsula Blood Bank

U. S. Simonds, Jr., was recently elected as president of the Peninsula Memorial Blood Bank, replacing Dr. Carl L. Hoag, who resigned July 1, 1961. Dr. Hoag has served as president of the blood bank since its inception in 1941. He will serve as consulting physician at the blood bank. The executive committee at its last meeting recommended that the life-time title

of honorary president be conferred upon Dr. Hoag.

Mr. Simonds initiated the idea of a local blood bank in 1941 and along with Dr. Hoag and Mrs. P. J. Hanzlik, present administrator, were influential in organizing the blood bank at its first meeting held at Mills Memorial Hospital. Mr. Simonds is executive secretary of the Building Trades Council and is a member of the board of trustees of the San Mateo Junior College District. He has been mayor and councilman of Burlingame and was named Burlingame's "Man of the Year" in 1953. He has been vice-president of the blood bank for 19 years. He will serve as president for the unexpired term of Dr. Hoag and until the annual meeting of trustees in January 1962.

Committee on Blood Bank Systems Trying to Define "Donor"

An Ad Hoc Committee on Blood Banking Systems in the United States composed of Mark F. Lesses, M.D., Chairman, LeRoy Bates, M.D., Ralph M. Hartwell, M.D., Bernice M. Hemphill, W. Quinn Jordan, Keith D. McMilan, M.D. (ex officio), and John R. Schenken, M.D. (ex officio), has met three times. The committee has classified donors into four categories: 1. The replacement donor. 2. The paid donor. 3. The assurance donor. 4. The donor who gives for any of several motives, the "voluntary" or "altruistic" donor. Discussions have been held concerning the roles played by the voluntary donor, replacement donor and paid donor. Next to be considered is the role and impact of the insurance donor. An attempt is being made to obtain information on benefits or defects of each type of donor from the standpoint of adequacy, cost, transmission of hepatitis, wastage, and reliability.

News and Announcements to be a Regular Feature

The *News and Announcements* section, which first appeared in the July-August issue of TRANSFUSION, will continue to appear regularly. Material received 30 days before press time may be incorporated in this section.

The editor of this section is Frank E. Trobaugh, Jr., M.D., Secretary of the AABB, who is assisted by Miss Lois James, Office Manager, AABB. This section provides a communications medium for news of general interest to blood bankers. Any information deemed suitable for general distribution to blood bank personnel will be considered appropriate for publication. Following is an outline of material considered suitable for inclusion in News and Announcements.

1. Committee Chairmen may wish to give interim reports designed to inform the membership on progress of programs sponsored by the AABB. This is felt to apply especially to National Committee on Clearinghouse, Inspection and Accreditation, Reference Laboratories, Standards, Education (Certification and Training), Workshops, Membership, and Finance.

2. Reports of State Representatives.

3. Reports by Presidents of State Blood Banking Associations, including announcements of future meetings. Programs should be included.

4. Reports of past meetings. When a meeting has already occurred the report should in essence be a brief summary of the activities and the contents of the papers given.

5. Actions and announcements by the Board of Directors of the AABB.

6. News of the Joint Blood Council, National Institutes of Health, National Research Council, Office of Civil Defense Mobilization, and American Red Cross as it may pertain to blood banking.

7. Announcements of training programs scheduled.

8. News of members: awards, grants, honors, changes of address and jobs.

9. Significant anniversaries and milestones in the lives of individuals as well as institutions.

10. Obituary notices.

11. Applications for membership or other activities requiring registration.

12. Announcements of accredited blood banks and technologists certified including requirements for qualification.

13. Announcements of new editions and revisions of various publications of the AABB.

14. Correspondence.

15. Central Office news.

Readers are encouraged to submit information deemed suitable for publication in News and Announcements to: Frank E. Trobaugh, Jr., M.D., Editor, News and Announcements, American Association of Blood Banks, 30 North Michigan Avenue, Chicago 2, Illinois.

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